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Attn: TSCA Docket Clerk

Re: For Your Information Submission:

The enclosed information is submitted on behalf of Dow Corning Corporation, Midland, Michigan, 48686-0994, on a For-Your-Information (FYI) basis as a follow-up to submissions made concerning octamethylcyclotetrasiloxane (OMCTS), which chemical substance was the subject of a health and safety data rule issued under Section 8(d) of the Toxic Substances Control Act (TSCA) and with an effective date of December 28, 1984 (sunset date December 28, 1994), as codified at 40 CFR 716 (Health and Safety Data Reporting). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

2002 DEC 18 PM 2:14

Listed Chemical Substance:

556-67-2 Octamethylcyclotetrasiloxane (OMCTS, D_4)

Final Study Report:

Non-Regulated Study: Effects of Octamethylcyclotetrasiloxane (D_4) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28 Day Inhalation Study

Dow Corning Corporation
2002-I0000-52111
November 21, 2002

FYI-02-001439

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84030000006

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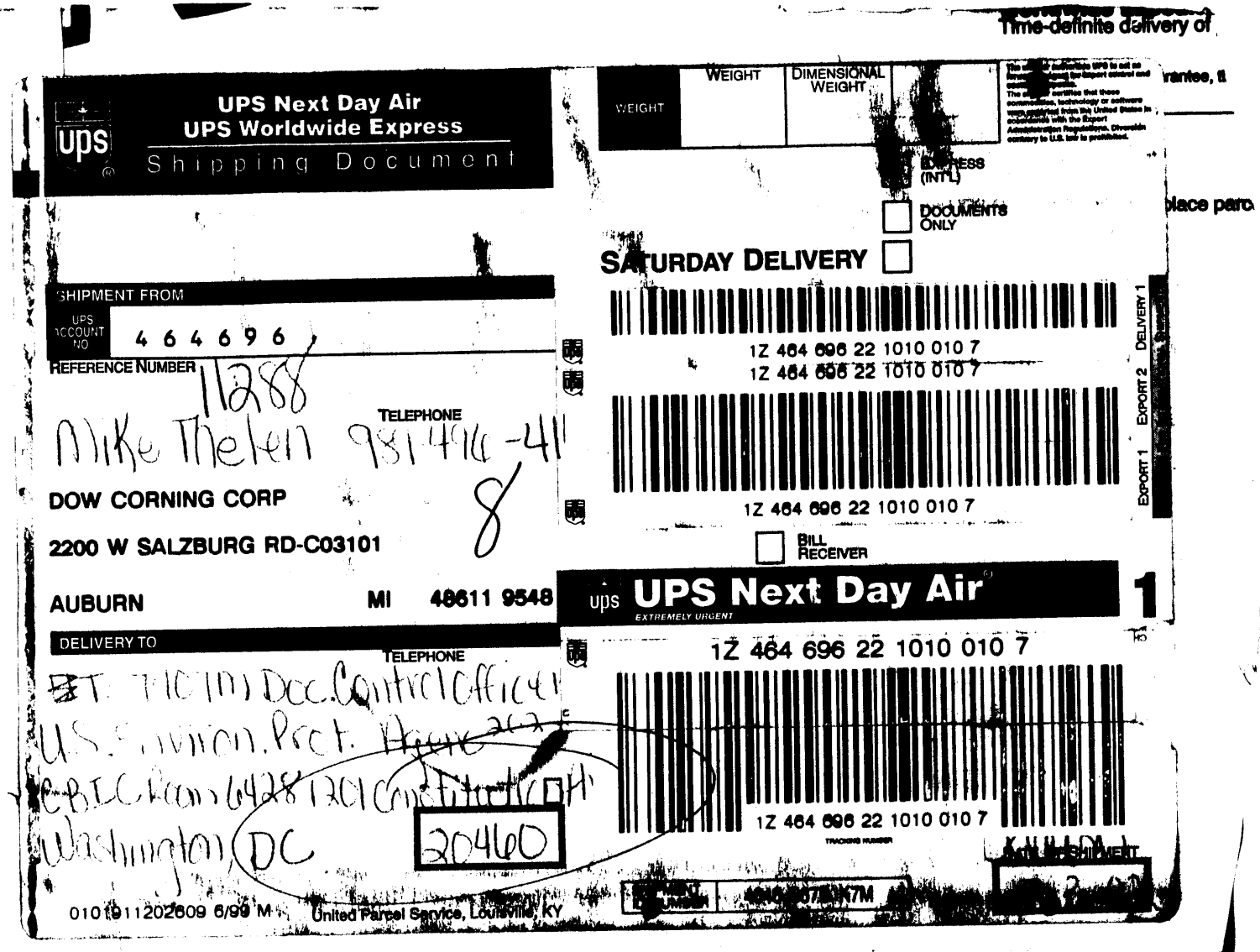
For purposes of this TSCA For-Your-Information (FYI) submission, the general INTERNAL designation on the attached health and safety report is waived by Dow Corning.

If you require further information regarding this submission, please contact Michael Thelen, Manager of U.S. EPA Regulatory Affairs, at 989-496-4168 or at the address provided herein.

Sincerely,

Kathleen P. Plotzke
Director, Health and Environmental Sciences
(989) 496-8046



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DOW CORNING CORPORATION
HEALTH & ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

Report No.:	2002-I0000-52111
Title:	**Non-Regulated Study: Effects of Octamethylcyclotetrasiloxane (D4) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28 Day Inhalation Study.**
Study No.:	8491
Test Article:	Octamethylcyclotetrasiloxane (D4)
Study Director:	Paul A. Jean, Ph.D. Senior Toxicology Specialist
Sponsor:	Dow Corning Corporation 2200 West Salzburg Road Auburn, Michigan 48611
Sponsor Representative:	Steven D. Crofoot, M.S. Team Leader, Toxicology
Testing Facility:	Dow Corning Corporation Health and Environmental Sciences 2200 West Salzburg Road Auburn, Michigan 48611
Study Completion Date:	November 21, 2002

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GLP Compliance Statement: The work described in this report was carried out using the best available scientific methodology, and procedures were followed to assure accurate, high quality results. However, this non-regulated study was ***not*** conducted to meet all of the requirements described in Good Laboratory Practices Regulations such as those documented in the Federal Register 40 CFR Part 792.

ABSTRACT

Octamethylcyclotetrasiloxane (D4) is a low molecular weight cyclic siloxane with both industrial and commercial applications. Repeated exposure to D4 vapor has been shown to induce a phenobarbital-like liver enlargement and induction of cytochrome P450 enzymes in rats. The primary objective of the present study was to evaluate the effects of exposure to D4 vapor on liver and thyroid cell proliferation and liver hypertrophy with respect to exposure concentration and duration. This study was performed in three distinct phases. Phase I was conducted using a known hepatotoxicant ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid) to develop the techniques required for evaluation of cell proliferation in phases II and III. Phase II was conducted to assess the temporal relationships regarding cell proliferation, hypertrophy and changes in organ weight following 1, 2 and 4 five-day exposure periods. Phase III was conducted to evaluate the dose-response relationships regarding cell proliferation, hypertrophy and changes in organ weight after one five-day exposure period. This exposure duration was chosen based on the peak hepatic response demonstrated in phase II. Female Fischer 344 rats were used in each of the three phases. In phases II and III rats were exposed via whole body inhalation to D4 vapor for 6 hr/day; 5 days/week for up to 4 weeks. A separate group of female Fischer 344 rats were provided drinking water containing phenobarbital (PB; 0.05% w:v) for 5 days/week. These rats served as a positive control group. Cell proliferation was measured by incorporation of 5'-bromo-2-deoxyuridine (BrdU) and by changes in the relative abundance of proliferating cell nuclear antigen (PCNA). Liver hypertrophy was determined by evaluating the number of nuclei present in a fixed microscopic field size relative to controls. Alza® osmotic mini pumps (2ML1, 10 µl/hr) containing BrdU (20 mg/ml) were implanted (subcutaneous) 6 days prior to necropsy. In phase II, ten animals from each treatment group (0 and 700 ppm D4, 0.05% PB) were euthanized on day 6, 13, and 27. In phase III, rats were exposed to 0, 7, 30, 70, 150, 300, or 700 ppm D4 vapor or 0.05% PB and euthanized on day 6. Liver and thyroid tissues were collected, weighed, sectioned, and processed for subsequent immunohistochemical analysis.

In phase II exposure to 700 ppm D4 increased liver weight (liver-to-body weight ratio) by 14(18), 19(19), and 20(22) percent relative to controls while PB treatment gave rise to increases of 36(33), 31(27), and 29(27) percent relative to controls on day 6, 13, and 27, respectively. Liver hypertrophy was observed in both D4 and PB exposed animals. Exposure to 700 ppm D4 decreased the number of nuclei per microscopic field by 9% on day 6, 15% on day 13 and 11% on day 27. This temporal relationship was similar to PB which induced a decrease in the number of nuclei per microscopic field by 18%, 19% and 26% on days 6, 13 and 27, respectively. Hepatic cell proliferation, as measured by BrdU incorporation, increased approximately 3-fold relative to control by day 6, returned to control levels by day 13, and was below control levels on day 27 in rats exposed to 700 ppm D4. This pattern of transient hyperplasia was observed in all hepatic lobes examined and was similar to that observed for PB-treated rats. The relative abundance of PCNA followed a similar profile as BrdU incorporation with the largest increase in PCNA expression observed on day 6 (approximately 4-fold over controls) and return to control levels or lower by day 27 for both D4- and PB-treated rats. Thyroid weight (thyroid to body weight ratio) was increased relative to control for D4-exposed rats by approximately 13(18), 18(19) and 15(18) percent on day 6, 13, and 27, respectively. In contrast, PB did not induce a significant change in thyroid weight on day 6. However, increases in thyroid weight (thyroid to body weight ratio) of 20(14)% and 27(25)% were present on day 13 and 27, respectively. Thyroid cell proliferation, as measured by BrdU incorporation, in D4-exposed animals was increased on days 6 (7-fold) and 13 (5-fold) and then returned to control levels by day 27. In contrast, increases in thyroid BrdU incorporation of 4-, 13- and 5-fold were present at day 6, 13, and 27 in PB-treated rats, respectively. In phase III liver weight (absolute and relative to body weight) increased in a dose-responsive manner. The lowest exposure concentration to yield a statistically significant increase in the liver weight (liver to body weight ratio) was 150 ppm (70 ppm) D4. In contrast to the results obtained in phase II, phase III liver hypertrophy was not observed after the single five-day exposure period. However, cell proliferation, as measured by hepatic BrdU incorporation, was observed and followed a dose-response pattern similar to that for liver weight with statistically significant increases in labeling at exposure concentrations of 70 ppm and higher. Unfortunately, a statistically significant increase in PCNA levels at 7 ppm but not 30 ppm limited interpretation of the relationship between D4 exposure concentration and hepatic PCNA levels. Thyroid weight and thyroid hyperplasia for D4- and PB-treated rats were not different from controls in this phase. The lack of a D4-induced increase in thyroid

weight and cell proliferation is contrary to that observed in phase II. The underlying cause for this discrepancy is not known, however variability in the time for developing measurable changes in the thyroid and the short duration of this study phase is suspected to be involved.

The results of this study demonstrate that repeated D4 vapor inhalation exposure can induce a transient dose-dependent cell proliferation and sustained hypertrophy in the liver of female rats. Even though the D4-induced changes in thyroid weight and cell proliferation observed in phase II following a single five-day exposure period did not repeat in phase III, the data suggest that continued exposure would promote a transient increase in thyroid cell proliferation and a sustained increase in thyroid weight. These organ changes are consistent with that expected of PB-like compounds.

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GLP COMPLIANCE STATEMENT

The work described in this report entitled **"Non-Regulated Study:Effects of Octamethylcyclotetrasiloxane (D4) On Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28 Day Inhalation Study"** (Dow Corning Corporation study number: 8491) was carried out using the best available scientific methodology, and procedures were followed to assure accurate, high quality results. However, this non-regulated study was ***not*** conducted to meet all of the requirements described in Good Laboratory Practices Regulations such as those documented in the Federal Register 40 CFR Part 792.

Paul A. Jean, Ph.D.
Study Director

21 NOV 02
Date

Steven D. Crofoot, M.S.
Team Leader, Toxicology

11/21/2002
Date

APPROVAL SIGNATURES

This report consists of pages 1 through 75 including Tables 1 through 17 and Appendices A through C.

Paul A. Jean 21Nov02

Paul A. Jean, Ph.D. Date
Study Director

Steven D. Crofoot 11/21/2002

Steven D. Crofoot, M.S. Date
Team Leader, Toxicology

STUDY INFORMATION

Study Initiation Date:	October 31, 1995
Experimental Start Date:	November 7, 1995
Experimental Termination Date:	May 1, 2000
Study Completion Date:	November 21, 2002
Study Director:	Paul A. Jean, Ph.D.
Sponsor:	Dow Corning Corporation 2200 W. Salzburg Road Auburn, Michigan 48611
Sponsor Representative:	Steven D. Crofoot, M.S. Team Leader, Toxicology

OBJECTIVE

The principle objectives of this study were to establish immunohistochemical methods for assessment of cell proliferation and then utilize the methods to evaluate the effects of repeated whole-body vapor inhalation exposure of female Fischer 344 rats to octamethylcyclotetrasiloxane (D4) on hepatic and thyroid cell proliferation and liver hypertrophy as a function of exposure concentration and duration.

TEST, CONTROL, REFERENCE ARTICLE INFORMATION

1. Test Article

A.	Identification:	Octamethylcyclotetrasiloxane (D4) Supplied as Dow Corning ® 244 Fluid
B.	Lot Number:	LL024 S10
C.	CAS Number:	556-67-2
D.	Physical Description:	Liquid
E.	Storage Conditions:	Ambient
F.	Handling Precautions:	See MSDS
G.	Stability: Expiration Date:	Stable (30 months) December 1996
H.	Purity:	99.6%
I.	Preparation of Dosing Solution:	NA
J.	Concentration of Solution:	NA
K.	Stability article in solution:	NA
L.	Supplier:	Dow Corning Corporation
M.	Analytical Procedure to Verify Test Article:	GC/TCD & GC/MSD
N.	Reserve Sample:	Yes

2. Positive Control Article

A.	Identification:	Phenobarbital sodium salt (PB)
B.	Lot Number:	122H0143
C.	CAS Number:	50-06-6
D.	Physical Description:	White powder
E.	Storage Conditions:	Ambient Conditions
F.	Expiration date:	6/1/99
G.	Stability:	Stable at room temperature
H.	Purity:	> 99%
I	Concentration of Dosing Solution:	0.05% in drinking water (w/v)
J.	Stability in Dosing Solution:	Stable over time stored and used
K.	Analytical Procedure to Verify Test Article:	None used
L.	Handling Precautions:	Wear impervious gloves, safety goggles, protective clothing. This material is a possible teratogen and effects the CNS.
M.	Supplier:	Sigma Chemical
N.	Reserve Sample:	Retention sample will be collected according to Standard Operating Procedures and stored in the Archives at Dow Corning Corporation.

3. Reference Article

A.	Identification:	Wyeth 14,643 [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid
B.	Lot Number:	CSL-91-314-100-33

C.	CAS Number:	50892-23-4
D.	Physical Description:	off-white powder
E.	Storage Conditions:	Ambient conditions
F.	Expiration Date:	10/2000
G.	Stability:	Stable
H.	Purity:	>98%
I.	Preparation of Dosing Solution:	Prepare in suitable solvent such as DMSO, diethyl acetate, or methylene chloride. Dilute into corn oil to 25 mg/ml. Dose 50 mg/2ml/kg ip.
J.	Concentration of Solution:	25 mg/ml
K.	Analytical Procedure to Verify Test Article:	NA
L.	Handling Precautions:	Gloves, lab coat, disposable respirator: handle as a carcinogen
M.	Supplier:	Chemsyn Science Laboratories
N.	Reserve Sample:	Yes

ROUTE OF EXPOSURE

In phase I Wyeth-14,643 (50 mg/kg) was administered once daily (ip) for 4 consecutive days. Phase I was conducted using Wyeth-14,643, a known hepatotoxicant, for method development to assess cell proliferation in phases II and III.

In phases II and III D4 was administered via whole body vapor inhalation exposure. Exposures were for 6 hr/day, 5 days/week for up to 4-weeks.

Phenobarbital was administered as a drinking water additive at a known tumor-promoting dose (0.05% w/v) in both phases II and III. Water consumption was monitored each time water bottles were changed in order to estimate the amount of phenobarbital consumed and to be sure that the presence of phenobarbital in the drinking water would not make the water less palatable.

TEST SYSTEM

Species: Rattus norvegicus

Strain: Fischer 344

Source: The Charles River Breeding Laboratories, Inc. Raleigh, North Carolina

Number on study:	**Phase I**	**Phase II**	**Phase III**
	8	90	80

Number Ordered:	**Phase I**	**Phase II**	**Phase III**
	9	100	90

Animals were ordered in three separate shipments, one per phase. A weight-stratified randomization procedure was used to order animals into treatment groups. Extra animals within each order allowed outliers to be removed from the study and thus provided each group with animals of similar weight.

Approximate Age at initiation of exposure: 8 to 11 weeks of age

Approximate Weight at initiation of exposure: 150 to 170 g

Quarantine Period: 7 days

Identification Method: Each animal received a Q# upon receipt. After randomization animals selected for use were uniquely identified by a Monel® metal ear tag displaying the animal number. Individual cage labels were used to display the animal number, group number, study number, exposure level, sex, species, strain, Study Director, exposure initiation date, and in-life phase termination date.

METHOD OF EUTHANASIA

All animals were anesthetized with CO_2 (approximately 20-30 seconds) and then exsanguinated.

JUSTIFICATION FOR SELECTION OF TEST SYSTEM

The Fischer 344 rat is recognized as an appropriate animal model for toxicity studies and represents a widely used strain for which significant historical data are available. In addition, the Fischer 344 rat has been used in previous studies of D4. Therefore it was appropriate to use this strain to maintain consistency and allow for cross study comparisons. Females were chosen over males because they have historically demonstrated greater degrees of liver enlargement following repeated inhalation exposure to D4 in subacute toxicity study designs. Ten animals (N=10) per group, for phases II and III, was determined to be sufficient based on power analysis and observations made in previous studies. The use of eight animals in phase I was considered appropriate for purposes of methods development.

HOUSING AND MAINTENANCE (HUSBANDRY)

1. Housing Requirement: All animals were individually housed in clean suspended wire-mesh cages (7" X 10" X 7") during the quarantine and in-life phase of the study. Before each exposure, animals were transferred into cages designed for placement within the exposure chambers. Following the exposure period, the animals were returned to their original cages. Cages used during non-exposure periods were elevated above Bed-O'Cobs® bedding. The bedding was changed at least twice a week. The cages were subjected to routine cleaning at a frequency consistent with the Animal Resource Department SOPs. During the in-life portion of the study the animals were located in the following animal rooms; room 215 (phase I), room 217 (phase II) and room 218 (phase III) within Dow Corning Corporation's Health and Environmental Sciences building.

2. Environmental Conditions: Environmental controls were set to maintain room temperature between 65-78°F and relative humidity at 40-70%. Air handling units were set to provide approximately 10-15 air changes per hour. Fluorescent lighting controlled by light timers provided illumination for a 12-hour light/dark cycle (~6:00 a.m.-6:00 p.m.). Temperature and relative humidity were monitored continuously and the light cycle was checked at least once a week.

3. Basal Diet: Purina® Certified Rodent Chow ® #5002 was offered *ad libitum* (except during chamber exposures) during the study. Analyses of the certified feed for the presence of nutritional components, heavy metals and pesticides, was performed and provided by the manufacturer. The low levels of contaminants detected were acceptable for the present study, based on the end points being measured.

4. Drinking Water: Reverse osmosis purified water was available *ad libitum* (except during chamber exposure) upon arrival at the testing facility and throughout conduct of the study. Water bottles were changed at least twice a week. Animals in groups 3A-3C and groups A8 and B8 of phases II and III, respectively, were administered phenobarbital in their drinking water. Water consumption was monitored to ensure that the animals receiving phenobarbital in their water found it to be palatable and were drinking normally.

ANIMAL RECIEPT AND QUARANTINE

Upon receipt, each animal was given a general health exam as it was transferred from the shipping carton to the quarantine cages. All study animals were quarantined for 7 days. During the quarantine period each animal was observed twice daily during the week, and once a day on weekends, for changes in general appearance and behavior.

ANIMAL WELFARE ACT COMPLIANCE

Animal use in this study was in compliance with all applicable sections of the final rules of the Animal Welfare Act regulations (9 CFR): parts 1, 2, and 3.

EXPERIMENTAL DESIGN

1. Randomization (all phases): Animals were weighed and randomized by weight into appropriate test groups upon release from quarantine. The randomization process utilized the animal weights and the random number generation features of Excel (Version 5.0) to assign individual animals to each group while maintaining group body weight homogeneity. After randomization, test animals were ear tagged and cage labels with group assignments and other pertinent data were placed on the outside of each cage.

2. Phase I (method development): Animals were randomized into two groups, group 1 (control) and group 2 (Wyeth). On study day 0, each animal was anesthetized with isoflurane and fitted with a mini osmotic pump. Each pump was filled with 5-bromo-2-deoxyuridine (BrdU; 20 mg/ml) and surgically placed under the skin for continuous subcutaneous (sc) administration. The rate of administration was 10 µl /hr or 20 µg/hr for 5 days. On study days 1-4 each animal was administered corn oil (group 1) or Wyeth (group 2), a hepatotoxicant known to cause liver hyperplasia.

Group	Treatment	Route	N
1	corn oil (2 ml / kg)	ip	4
2	Wyeth (50 mg /kg)	ip	4

Necropsy was conducted on study day 5. Animals were euthanized by CO_2 asphyxiation. Liver, lungs, brain and a section of duodenum was collected from each animal. Liver, lung and brain weights were taken. The section of duodenum, and one section from the median, left and right lateral lobes of the liver were placed in 10% formalin for histologic evaluation. The remaining portion of liver, lungs and brain were frozen in liquid nitrogen and then stored at approximately -80°C. An assessment of cell proliferation was then conducted on the fixed liver and duodenum tissues following standardized immunohistochemical methodologies (BrdU staining and PCNA analysis). Body weights were taken at randomization, study day 0 and study day 5.

The activities conducted within phase I represent method development focused on developing the skills and establishing the methodologies required to assess cell proliferation.

3. Phase II: Animals were randomized into three groups, each with three subgroups as follows, group 1A-1C (controls), group 2A-2C (700 ppm D4), and group 3A-3C (0.05% PB). On study day 0, 7, and 21 subgroup "A", "B", and "C" animals, respectively, were anesthetized with isoflurane, prepped for aseptic surgery, and fitted with a mini osmotic pump. Each pump was filled with BrdU (20 mg/ml) and surgically placed under the skin for continuous sc administration of BrdU. Exposure to D4 (groups 1A-1C and 2A-2C) and PB (groups 3A-3C) began for all animals on study day 1. The exposure period consisted of 1, 2,

and 4 five-day exposure periods (6 hour/day) for animals in subgroups "A", "B" and "C", respectively. Two non-exposure days separated each successive five-day exposure period. The large number of animals per group required the use of two exposure chambers per exposure level. On study day 6, 13, and 27 subgroup "A", "B", and "C" animals were euthanized, respectively. Blood was collected from the abdominal aorta of anesthetized animals (CO_2 inhalation) followed immediately by euthanasia via exsanguination. Liver, lungs, brain, thyroid and a section of duodenum were collected from each animal. Organ weights were determined for the liver, lungs, brain and thyroid (after fixation). The thyroid/parathyroid, lungs, duodenum section and one section from the median, left and right lateral lobes of the liver were placed in 10% formalin for histologic evaluation. The remaining portion of liver and the brain were frozen in liquid nitrogen and then stored at approximately -80°C. An assessment of cell proliferation was then conducted on the fixed tissues (liver and thyroid) following standardized immunohistochemical methodologies (BrdU staining and PCNA analysis). Liver hypertrophy was evaluated microscopically. Body weight and consumption of food and water were monitored weekly. Clinical observations were conducted daily. The blood sample collected from each animal was not evaluated. The remaining liver samples and brain of each animal collected at necropsy was not evaluated. The lungs collected from each animal at necropsy were processed into slides and stained for BrdU analysis. No staining was observed in the preliminary evaluation of the slides. The lungs were not evaluated further.

Group/subgroup	Treatment	Route	N
1A	Air	Inhalation	10
1B	Air	Inhalation	10
1C	Air	Inhalation	10
2A	700 ppm D4	Inhalation	10
2B	700 ppm D4	Inhalation	10
2C	700 ppm D4	Inhalation	10
3A	PB	Drinking water	10
3B	PB	Drinking water	10
3C	PB	Drinking water	10

4. Phase III: Animals were randomized into eight groups (1-8), each with two subgroups (A and B). Group 1 animals served as controls (air only exposure). Animals in groups 2-7 were exposed to D4 vapor at concentrations ranging from 7 to 700 ppm and animals in group 8 were exposed to PB (0.05% PB in the drinking water). The large number of animals on study required that this phase be conducted in two parts, the second representing a repeat of the first. Subgroup "A" animals were used in the first part and subgroup "B" animals in the second. Within each part animals were anesthetized with isoflurane, prepped for aseptic surgery, and fitted with a mini osmotic pump on study day 0. Each pump was filled with BrdU (20 mg/ml) and surgically placed under the skin for continuous subcutaneous administration of BrdU. Exposure to D4 (groups 2-7) and PB (group 8) began on study day 1. The exposure period consisted of a 5-day period of exposure. On study day 6 the animals were anesthetized by CO_2 inhalation and then euthanized by exsanguination. Liver, lungs, thyroid/parathyroid and a section of duodenum were collected from each animal. Organ weights were determined for the liver and thyroid (after fixation). The lungs, duodenum section and one section from the median, left and right lateral lobes of the liver were placed in 10% formalin for histologic evaluation. An assessment of cell proliferation was conducted on the liver (BrdU and PCNA) and thyroid (BrdU) following standardized immunohistochemical methodologies. Liver hypertrophy was evaluated microscopically. Body weight and consumption of food and water were monitored weekly. Clinical observations were conducted daily.

Group/subgroup	Treatment	Route	N
1A	Air	Inhalation	5
1B	Air	Inhalation	5
2A	7 ppm D4	Inhalation	5
2B	7 ppm D4	Inhalation	5
3A	30 ppm D4	Inhalation	5
3B	30 ppm D4	Inhalation	5
4A	70 ppm D4	Inhalation	5
4B	70 ppm D4	Inhalation	5
5A	150 ppm D4	Inhalation	5
5B	150ppm D4	Inhalation	5
6A	300 ppm D4	Inhalation	5
6B	300 ppm D4	Inhalation	5
7A	700 ppm D4	Inhalation	5
7B	700 ppm D4	Inhalation	5
8A	PB	Drinking water	5
8B	PB	Drinking water	5

TEST ARTICLE PREPARATION AND ANALYSIS

Wyeth 14,643 (also known as [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid) (approximately 250 mg) was first dissolved into approximately 10 ml ethylacetate with stirring. This solution was then added to 10 ml corn oil (37°C) and allowed to mix with stirring over night. The mixture was warmed and the vial open to the atmosphere to allow ethyl acetate to evaporate. This evaporation process was completed when the solutions volume was reduced to approximately 10 ml. Wyeth was used as provided and dosing solutions were not evaluated for stability or confirmation of nominal concentration.

Octamethylcyclotetrasiloxane vapor generation was performed following standard methodologies. Test article was metered into a heated glass J-tube for vaporization. Carrier gas (filtered air) was passed at a controlled rate through the J-tubes. The air/vapor mixture exiting the J-tube was directed into the inlet port at the top of the 450-Liter Rochester style chambers where it mixed with chamber make-up air to achieve target chamber vapor concentrations. Animal positions within the exposure caging were rotated on a daily basis to minimize the potential effect of any concentration variations in the chambers. Further details regarding inhalation exposure methodology are provided in appendix B.

Phenobarbital drinking water solutions (0.05% weight:volume) were prepared based on weights and volumes in sufficient quantity to provide water to animals for the duration of the experimental phase. In phase II, fresh solutions were prepared weekly. The solutions were prepared using reverse osmosis purified water and stored in an appropriate container at $5^{\circ} \pm 3^{\circ}C$. Phenobarbital was used as provided and dosing solutions were not evaluated for stability or confirmation of nominal concentration.

PROCEDURES

1. Preparation of BrdU Stock Solution: 5-Bromo-2-deoxyuridine was purchased from Sigma Chemical Company (catalog number: B-5002). BrdU is light sensitive, and therefore was protected from fluorescent light exposure. All work was performed under incandescent lighting. Storage of stock BrdU powder was at 5° ± 3°C desiccated. To prepare the working stock, BrdU powder was removed from the refrigerator and allowed to warm to room temperature. BrdU powder was weighed and dissolved in sterile phosphate buffered saline (pH 7.6±0.1) in an amount that would yield a final concentration of approximately 20 mg/ml. The PBS solution was prewarmed to 37-40°C in some instances to facilitate the solubilization of BrdU. Stirring or shaking the mixture also helped facilitate the process.

2. Surgical Placement of Mini Osmotic Pumps: Osmotic pumps (model 2ML1, 7 day) were obtained from Alza Corporation, Palo Alto, CA. The pumps for a given experiment were all of the same type and lot. Individuals trained in aseptic techniques performed the subcutaneous implantation of the mini pumps. All surgical procedures as well as loading of pumps were done under aseptic conditions. The animal was anesthetized (isoflorane vapor) and the skin was prepared at the site of implantation by shaving an area on the upper back close to the neck and then washing the area with betadine and/or alcohol swabs. An incision was made perpendicular to the spine and the pump inserted just under the skin with the BrdU delivery end nearest the head. The skin around the incision was carefully pulled back together and closed with wound clips and/or sutures.

3. Tissue Processing: When all samples were collected and placed in fixative, the fixation containers were placed on a shaker for at least 4 hours. Note that the total time for fixation (shaker time plus non-shaker time) did not exceed 45-55 hours. Following formalin fixation, the tissues were processed as follows: 2-changes of 30% ethanol, 1-change of 70% ethanol, 2-changes of 80% ethanol, 2-changes of 95% ethanol, 2-changes of 100% ethanol, and 2-changes of xylene. Actual times and subsequent processing steps were done according to standard operating procedures. Samples were embedded in paraffin blocks as soon as possible. A low melt paraffin was used.

Paraffin embedded tissues were sectioned and placed on glass slides for immunostaining or staining with hematoxylin and eosin.

4. Immunohistochemical Staining Procedures: Visualization of BrdU and PCNA antigens was accomplished with the DAKO EnVision® System Peroxidase according to the manufacturer's procedures. This detection method uses a horseradish peroxidase (HRP) labeled polymer attached to secondary antibody that recognizes primary antibodies produced in either rabbit or mouse. Tissue sections were deparaffinized in xylene and taken to water via graded alcohols.

For PCNA detection, the deparaffinized section was placed in a coplin jar with 35-45 ml of a target unmasking fluid (TUF) (Signet Laboratories, Dedham, MA). The coplin jar was then placed into a 600 ml beaker containing 300-400 ml of water and heated in a microwave for 1-3 min. Care was taken to insure that the TUF solution did not boil. The beaker containing the coplin jar was then cooled in a beaker with running tap water for 20-40 min. The tissues were then rinsed two times (5-10 min each change) with phosphate buffered saline containing 0.1% Tween-20 (PBSt). Peroxidase blocking agent was added for 5 min followed by two rinses with PBSt. Primary antibody for PCNA was diluted 1:100 in phosphate buffered saline with 1% bovine serum albumin (BSA) and applied to the sections. Following two more rinses with PBSt the labeled polymer was applied and allowed to incubate for 10 min. This was followed with two washes with PBSt (3-6 min each) prior to incubating with substrate-chromagen (in the dark) for 5 min. Two more PBSt washes (3-6 min) prepared the tissues for the DAB enhancer which was applied for 5 min. The sections were then rinsed with two changes of reverse osmosis purified water (3-6 min each change) prior to being counter stained in aqueous hematoxylin (Innovex Biosciences, Richmond, CA). The sections were then dehydrated by incubations with 50%, 95%, and 100% (absolute) ethanol. The slides were cleared with two rinses in xylene (5-10 min each) and cover slipped.

For detection of BrdU, tissue DNA must be denatured to facilitate antigen interaction with the primary antibody. Thus, sections were incubated in 4N HCl at 37°C for 20-25 min and then rinsed with reverse osmosis purified water. Subsequent steps were as described above for PCNA detection. The primary antibody recognizing BrdU was diluted 1:200 in PBS with BSA (1%) just prior to use.

Cell replication was determined by counting the number of positively stained hepatocytes with the BioQuant® Image analysis system (Bioquant-R & M Biometrics, Inc., Nashville, TN) under 40X magnification. Portions counted were randomly selected and at least 1000 hepatocytes were counted per slide. The labeling index (LI %) was calculated by dividing the number of positive nuclei by the total number counted and then multiplying by 100.

IN-LIFE TEST SYSTEM OBSERVATIONS AND PROCEDURES

1. Food Consumption: In study phases II and III food consumption was monitored once per week. This was done by weighing the full feeder of each animal on day 1 of exposure and then re-weighing the feeder on the morning following the last exposure day of that week. The difference in feeder weights represents the amount of food in grams consumed for each animal over the experimental period.

2. Water Consumption: Water consumption was determined twice weekly on Monday and Thursday during study phases II and III. Water bottles from animals subject to necropsy were also weighed on the day of necropsy.

3. Body Weights: Individual body weights were taken prior to the start of the study for randomization into groups. During the in-life portion of the study, animals in phase I were weighed prior to dosing and at necropsy. Animals in phase II, groups 1A-3A had body weights taken on days 0 and 6. Groups 1B-3B body weights were taken on days 0, 7, and 13. Groups 1C-3C body weights were taken on days 0, 7, 14, 21 and 27. In phase III, animal body weights were taken on the day of pump implantation (day 0) and then again on the day of necropsy (day 6) for each subgroup.

In some instances body weights were determined with pumps in place. These body weights were adjusted by subtraction of the loaded pump weight.

4. Mortality/ Morbidity Checks: Animals were observed twice a day during the week and once a day on weekends and holidays for signs of mortality/morbidity by animal care personnel.

5. Clinical Observations: Clinical observations were made on each animal daily, including Saturdays and Sundays.

STATISTICAL METHODS

All data analysis was carried out using SAS® version 8.2. In all comparisons, the family wise error rate was held at 5% ($\alpha = 0.05$).

In phase I, the organ cellular proliferation data and body and organ weight data were analyzed using a one-way analysis of variance. Because there were only two treatment groups, a significant global F-test indicated a significant difference between the two treatment groups and no comparison of means was required. The Wilcoxon test was used in any situations where the data were not normally distributed or the variances were not equal among the groups, since there were only two groups.

In phase II, the organ cellular proliferation data were first evaluated with a three way analysis of variance (main effects: chamber, treatment, and day) to determine if there were significant differences within groups due to the use of more than one chamber per exposure level. In those cases where chamber was a significant effect, individual contrasts were constructed to compare the mean response within each of the two chambers in each treatment group. The cellular proliferation data, organ and body weight data and

food and water consumption data were then analyzed using a two-way analysis of variance (main effects: treatment and day). Where significant day*treatment interactions existed, the final analysis was a separate one-way analysis of variance (main effect: treatment) for each day. When the global F-test indicated a significant treatment effect, Dunnett's test for multiple comparisons against the control was used to compare the mean response in animals treated with either D4 or phenobarbital to the mean response in control animals. This method was used instead of Tukey's test for all possible comparisons because there was no interest in the comparison of the mean response in animals treated with D4 to the mean response in animals treated with the intra-assay positive control (phenobarbital) and because Dunnett's test is more powerful for determining a treatment effect under this design.

In phase III, organ cellular proliferation data, organ and body weight data and food and water consumption data from the control animals and those treated with D4 were analyzed with a one-way analysis of variance (main effect: dose). When the global F-test indicated a significant treatment effect, Dunnett's test for multiple comparisons against the control was used to compare the mean response in animals in each dose group against the mean response in animals in the control group. This test was used instead of sequential t-tests because it uses the pooled estimate of the variance, which is the best estimate and is a critical factor in controlling Type I error, and because this procedure properly accounts for the correlation among tests due to the fact that they are all made against a common control group.

The statistical analysis results are summarized in Appendix A.

RESULTS

Phase I:

Phase I of this study was designed to establish the technical skills and methodologies associated with BrdU and PCNA tissue handling, processing, and immunohistochemical evaluations. The data shown in tables 1 and 2 summarize the results of these experiments. Daily administration of Wyeth 14,643 for four consecutive days resulted in a significant increase (approximately 37%) in liver weight and liver-to-body weight ratio without significantly affecting body weight (Table 1). The effect on liver weight accompanied a significant increase in the LI for PCNA (4.2-fold) and BrdU (7.5-fold) (Table 2). These results demonstrate that pump placement, tissue collection and processing, immunohistochemical procedures, and image analysis methodologies were performed satisfactorily.

Phase II:

Phase II was conducted to evaluate the effect of exposure to 700 ppm D4 vapor on cell proliferation (liver and thyroid) and hypertrophy (liver) as a function of exposure duration. In this phase female Fischer 344 rats were exposed to 695, 696 and 700 ppm D4 vapor for 1, 2, or 4 five-day exposure periods, respectively. Note that each five-day exposure period was separated by a two-day non-exposure period. Control (air exposed) and PB-treated rats were placed into inhalation chambers to mimic the exposure routine of the D4 exposed animals. However, D4 vapor was not generated into these chambers. Summary data related to the inhalation exposure of animals in phase II is located in appendix B.

Rats exposed to 700 ppm D4 had an increased incidence of urine staining in the perineal region and the presence of a clear fluid around the eyes (Table 3). Phenobarbital treatment gave rise to an increased incidence of porphyrin staining around the eyes (Table 3). Exposure to 700 ppm D4 had no affect on food and water consumption over any of the exposure periods as shown in table 4. Exposure to 700 ppm D4 resulted in a 4% decrease in body weight after a single five-day exposure period (Table 5). In contrast, PB-treated rats demonstrated statistically significant increases in food and water consumption for each of the 1, 2, and 4 five-day treatment periods and a 4% increased in body weight at the end of the second five-day treatment period (Tables 4 and 5).

In Phase II exposure to D4 resulted in increased liver weight and liver-to-body weight ratios following 1, 2, and 4 five-day exposure periods (Table 6). The increases in liver weight (liver-to-body weight ratio) were 14(18), 19(19) and 20(22) percent greater than control after 1, 2 and 4 five-day exposure periods, respectively. Similarly, exposure to D4 resulted in increased thyroid and thyroid-to-body weight ratios following 1, 2, and 4 five-day exposure periods (Table 7). The increases were 13(18), 18(19) and 15(18) percent greater than control thyroid weight (thyroid-to-body weight) after 1, 2, and 4 five-day exposure periods, respectively. Note however that the 13% increase in thyroid weight was not statistically significant and that the 4% decrease in body weight after the first five-day exposure period likely contributed to the relative organ weight achieving statistical significance.

Accompanying the D4-induced increase in liver weight was an increase in hyperplasia as demonstrated by increases in the labeling indices for both BrdU and PCNA analysis (Table 8). The BrdU LI was increased approximately 3-fold after a single five-day exposure period in each of the three lobes evaluated. After 2 and 4 five-day periods of exposure to 700 ppm D4 the BrdU LI was not statistically different from control except for a 37% decrease in the median lobe at the end of 4 five-day exposure periods. The PCNA analysis in the median lobe gave similar results with LI increases of 4- and 2-fold after 1 and 2 five-day exposure periods, respectively. The PCNA LI was not different from control after 4 five-day exposure periods. One unexpected finding was a time-dependent increase in BrdU (2- to 3-fold) and PCNA (1.5 fold) labeling indices in the control animals. This change is believed attributable to the growth and hormonal changes associated with normal aging of female rats within the young adult-adult age range (8-13 weeks).

Exposure to PB in phase II resulted in increased liver and liver-to-body weight ratios following 1, 2, and 4 five-day exposure periods (Table 6). The increases were 36(33), 31(27) and 29(27) percent greater than control liver weight (liver-to-body weight ratio) after 1, 2, and 4 five-day exposure periods, respectively. Thyroid and thyroid-to-body weight ratios were increased after 2 and 4 five-day exposure periods (Table 7). The increases were 20(14) and 27(25) percent greater than control values after 2 and 4 five-day exposure periods, respectively.

Accompanying the PB-induced increase in liver weight was an increase in hyperplasia as demonstrated by increased labeling indices for both BrdU and PCNA analysis (Table 8). In each of the three lobes evaluated the BrdU LI was increased 4-5-fold after a single five-day treatment period. There was no difference in BrdU LI after 2 five-day treatment periods and a significant decrease (approximately 60% relative to control) in BrdU LI after 4 five-day treatment periods. Similar results were obtained with PCNA analysis of the median lobe. The PCNA LI after 1, 2, and 4 five-day treatment periods were 4-fold greater, 2-fold greater and 41% lower than control values, respectively.

Liver hypertrophy, as measured by a reduction in the number of nuclei within a fixed microscopic field size, was demonstrated for both D4 and PB exposed rats (Table 9). The mean number of nuclei per field was reduced by 9%, 15%, and 11% in rats following 1, 2, and 4 five-day D4 exposure periods, respectively. The 11% decrease after a 4 five-day exposure period did not achieve statistical significance. Similarly the mean number of nuclei per field was reduced by 18%, 19%, and 26% in rats following 1, 2, and 4 five-day PB treatment periods, respectively.

Thyroid cell hyperplasia was increased by D4 and PB (Table 10). Exposure to 700 ppm D4 for 1 and 2 five-day exposure periods was shown to cause a statistically significant 7- and 5-fold increase in BrdU LI, respectively. The BrdU LI after 4 five-day D4 exposure periods was not different from control. Exposure to PB for 1, 2, and 4 five-day exposure periods resulted in a 4-, 13-, and 5-fold increase in BrdU LI, respectively.

Phase III:

Phase III was conducted to evaluate the dose-responsiveness of hyperplasia (liver and thyroid) and hypertrophy (liver) after a single five-day exposure period. In phase III mean exposure concentrations (standard deviation) were 7(0), 29(1), 70(3), 150(5), 300(8), and 701(12) ppm D4 for target chamber concentrations of 7, 30, 70, 150, 300, and 700 ppm D4 vapor, respectively. Additional details regarding conduct of the inhalation exposures are provided in appendix B. As in phase II, exposure to D4 gave rise to an increased incidence in urine staining in the perineal region at 700 ppm D4 and an increased incidence of porphyrin staining around the eyes in PB-treated rats (Table 11). Exposure to D4 had no affect on food and water consumption with the exception of a 13% decrease in water consumption at the 700 ppm exposure level (Table 12). Body weight was similarly unaffected except for a 4% decrease at the 700 ppm exposure level (Table 12). Food and water consumption and body weight were increased by PB-treatment by 15%, 25% and 3%, respectively (Table 12).

Exposure to D4 resulted in statistically significant increases in liver weight and liver-to-body weight ratios at exposure concentrations equal to or greater than 150 and 70 ppm, respectively (Table 13). The increases in liver weight (liver-to-body weight ratio) were 7(6), 10(8), 13(12), and 13(18) percent greater than control for exposure concentrations of 70, 150, 300 and 700 ppm D4, respectively. Thyroid and thyroid-to-body weight ratios were not different from control following exposure to D4 in this phase (Table 14).

Accompanying the D4-induced increase in liver weight was a dose-related increase in hyperplasia as demonstrated by statistically significant increases in the labeling indices for both BrdU and PCNA (Table 15). This phase evaluated only the median lobe of the liver, a decision based on the results from phase II that demonstrated similar increases in hyperplasia across lobes. A statistically significant increase in BrdU LI was observed for exposure to 70 (2-fold), 150 (3-fold), 300 (3-fold) and 700 (4-fold) ppm D4. Similarly, statistically significant increases in PCNA LI was observed at exposure concentrations of 70 (2-fold), 150 (3-fold), 300 (4-fold) and 700 ppm D4 (4-fold). A statistically significant increase in PCNA LI was also obtained for rats in the 7ppm D4 exposure group (2-fold) but not for rats exposed to 30 ppm D4.

Administration of PB resulted in increased liver and liver-to-body weight ratios following a single five-day exposure period (Table 13). The increases were 29% and 25% greater than control values for liver weight and liver-to-body weight ratio, respectively. Thyroid weight (12%) and thyroid-to-body weight ratio (10%) were increased relative to control. However, these increases were not statistically significant (Table 14).

Accompanying the PB-induced increase in liver weight was an increase in hyperplasia as demonstrated by increased labeling indices for BrdU and PCNA (Table 15). The BrdU LI was increased 4-fold and the PCNA LI was increased 7-fold after one five-day treatment period.

Liver hypertrophy, as measured by a reduction in the number of nuclei within a fixed microscopic field, was not observed for D4-exposed or PB-treated rats in contrast to the results obtained in phase II (Table 16). The mean number of nuclei per field for the D4 and PB treated animals was not statistically different from the control values at any of the exposure concentration levels.

Thyroid cell hyperplasia, as measured by BrdU LI, was not evident following exposure to either D4 or PB in this phase (Table 17). This result is contrary to the 7-fold increase in BrdU LI that was observed in phase II with 700 ppm D4.

DISCUSSION

Repeated exposure to D4 has been shown to produce a reversible liver enlargement and a cytochrome P450 enzyme induction profile similar to that induced by PB in rats (McKim *et al.*, 1998). As a result D4 has been described as PB-like with respect to hepatic biochemical effects. In order to extend our understanding of the biochemical effects of repeated exposure to D4 in the rat, the present study has examined the effects of D4 on liver and thyroid hyperplasia and liver hypertrophy with respect to exposure duration and vapor concentration.

Organ hyperplasia can be measured by determining 1) the LI derived from an assessment of recent DNA replication or enumeration of cells in the S phase of the cell cycle, 2) by the mitotic index involving enumeration of the number of cells in mitosis, or 3) by the proliferative index representing changes in cell numbers (Goldsworthy *et al.*, 1995). Although all have utility, the method for determination of the LI (Eldridge *et al.*, 1990) was selected because of its sensitivity and relative simplicity. The LI was evaluated by two methods, incorporation of BrdU into DNA and the relative abundance of nuclear PCNA. As a nucleoside homolog, BrdU can be incorporated into DNA during DNA replication. The incorporated BrdU provides an immunohistochemical tag allowing identification and enumeration of cell proliferation. PCNA is an endogenous protein that participates in the control of the cell cycle (Bravo and MacDonald-Bravo, 1987). It is possible to determine which phase of the cell cycle a cell is in based on the staining intensity of PCNA.

Repeated vapor inhalation exposure of female Fischer 344 rats to 700 ppm D4 in study phase II resulted in an increase in liver weight (absolute and relative to body weight) of approximately 20% relative to control. Exposure duration (1, 2, or 4 five-day exposure periods) had little impact on the increase in liver weight. The effect of D4 exposure on liver weight was shown to be very dose-responsive in phase III. Statistically significant increases in liver weight were demonstrated for exposure concentrations above 70 ppm D4 for absolute liver weight and above 30 ppm D4 for liver:body weight ratio. Phenobarbital treatment resulted in significant increases in liver weight that were, like D4, sustained over the course of 4 five-day exposure periods.

The D4-induced increase in liver weight accompanied increases in hepatic cellular proliferation as demonstrated by increases in both BrdU and PCNA labeling indices. The data from phase II indicated that a burst of cellular proliferation occurred within the first five-day exposure period. The increase in hepatic cellular proliferation was not sustained with continued exposure to D4. This "burst" in cell proliferation was also observed in PB-treated animals and is characteristic of PB in mice and rats (Counts *et al.*, 1996; Peraino *et al.*, 1971). The D4-induced hepatic hyperplasia was demonstrated to be dose-responsive in phase III. Statistically significant increases in hepatic cellular proliferation were demonstrated for exposure concentrations above 30 ppm D4 for both markers, BrdU and PCNA. However, a 2-fold increase in PCNA LI was observed at the 7 ppm D4 exposure level. This increase is greater than that observed for both the 30 and 70 ppm D4 exposure groups and is greater than that for BrdU at 7 ppm D4. With exception to the finding at 7 ppm, the PCNA data in study phase III is in good agreement with the results obtained with BrdU. However, the equivocal LI results preclude interpretation of the PCNA data.

Repeated exposure to 700 ppm D4 in phase II gave rise to a sustained hepatic hypertrophy. Hypertrophy was apparent after 1, 2, and 4 five-day exposure periods. Sustained hepatic hypertrophy was also demonstrated for PB. The relationship to D4 vapor concentration could not be discerned because hypertrophy was not observed in D4 exposed rats in phase III. Interestingly, PB treatment did not induce hepatic hypertrophy in phase III. The factor(s) responsible for the lack of phase-to-phase reproducibility is not known. However, the other hepatic measures (liver weight changes and hyperplasia) were similar between the two phases.

The effect of D4 exposure on thyroid weight was evaluated. In study phase II exposure to 700 ppm D4 for 1, 2, and 4 five-day exposure periods resulted in a marked and sustained increase in thyroid weight (13-

19%). Phenobarbital treatment resulted in a marked and sustained increase in thyroid weight after 2 and 4 five-day treatment periods. These data suggest that D4 and PB can cause a sustained increase in thyroid weight with repeated exposure. The apparent differential response in phase II relative to phase III suggests that the effect of D4 and PB on thyroid weight is variable with regard to time of expression and that the peak response is best evaluated after more than one five-day exposure period.

Thyroid hyperplasia was evaluated in phase II to determine the temporal characteristics in response to repeated inhalation exposure to 700 ppm D4 and in phase III to determine the dose-responsiveness of the effect. The data obtained in phase II suggested that the peak response occurred soon after initiating repeated exposures (i.e. before the end of the second week of exposure). Unfortunately, as observed for thyroid weight, the one-week exposure to D4 in phase III did not result in an increase in thyroid cell proliferation. This was also true for phenobarbital treated rats. That 700 ppm D4 and phenobarbital treatments did not produce significant thyroid hyperplasia in phase III when measurable responses were present in phase II suggests the this response is variable with respect to time of expression and that the peak response is best evaluated after more than one five-day exposure period.

CONCLUSION:

The results of this study have demonstrated that repeated-exposure of female Fischer 344 rats to D4 can induce a sustained increase in liver weight (hepatomegaly), a rapid and non-sustained burst of hepatic hyperplasia, and sustained hepatic hypertrophy. This pattern of hepatic changes was similar to that observed with PB. The data support a 30ppm NOEL for D4-induced liver weight increases and hepatic cellular proliferation following a single five-day exposure period.

The effect of D4 exposure on the thyroid remains, in part, equivocal. The data from study phase II demonstrate that repeated-exposure to PB and D4 can yield a sustained thyroid weight increase and a non-sustained increase in hyperplasia. The timing of peak thyroid hyperplasia during the course of repeated exposure to D4 was not well-defined.

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Table 1.

Phase I: Effect of Wyeth 14,643 Exposure on

Body Weight and Liver Weight in Female Fischer 344 Rats

Treatment[1]	Terminal Body Weight[2] (g)	Liver Weight[2] (g)	Liver:Body Weight[2] (g)	N =
Corn oil	163.3 (6.6)	5.88 (0.27)	0.0361 (0.0019)	4
Wyeth 14,643	161.6 (3.7)	8.05* (0.34)	0.0498* (0.0012)	4

[1]Administered by intraperitoneal injection (corn oil at 2 ml/kg; Wyeth at 50 mg/2ml/kg)

[2]Values represent the mean (standard deviation)

*Statistically different from control ($p<0.05$)

Table 2.

Phase I: Effect of Wyeth 14,643 Exposure on Liver hyperplasia as Measured by BrdU and PCNA Immunohistochemistry in Female Fischer 344 Rats

Treatment[1]	Immunohistochemical Marker	Mean LI[2]	Standard Deviation	N =
Corn oil	BrdU	4.42	2.48	4
Corn oil	PCNA	2.18	1.73	4
Wyeth 14,643	BrdU	33.29*	8.79	4
Wyeth 14,643	PCNA	9.22*	6.26	4

[1]Administered by intraperitoneal injection (corn oil at 2 ml/kg; Wyeth at 50 mg/2ml/kg)

[2]LI = labeling Index (%)

*Significantly different from control at $p<0.05$

Table 3.

Phase II: Clinical Observations Summary

Clinical Observation	Incidence[1]		
	Control	700 ppm D4	0.05% PB
Normal	15 / 30	9 / 30	16 / 30
Urine Staining, perineal region	14 / 30	21 / 30	10 / 30
Porphyrin Staining, eye(s)	0 / 30	2 / 30	8 / 30
Clear Fluid, eye(s)	1 / 30	8 / 30	0 / 30
Dried Fluid, eye(s)	0 / 30	1 / 30	0 / 30
Eye(s), small	0 / 30	2 / 30	0 / 30
Eye(s), closed	0 / 30	1 / 30	0 / 30

[1]Incidence is expressed as " the number of animals expressing the finding at least once during the study period / total number of animals in the treatment group"

Table 4.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Food and Water Consumption

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	Food Consumption	12.2 (0.9)	12.3 (0.4)	12.7 (0.7)
700 ppm D4	Food Consumption	11.9 (1.1)	11.6 (1.7)	12.5 (0.5)
PB (0.05%)	Food Consumption	14.5* (1.3)	13.9* (0.8)	13.5* (0.8)
Control	Water Consumption	15.0 (1.2)	15.3 (2.5)	14.8 (1.5)
700 ppm D4	Water Consumption	14.6 (2.4)	14.3 (1.5)	16.2 (2.4)
PB (0.05%)	Water Consumption	20.8* (1.3)	18.2* (0.7)	17.7* (0.9)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent consumption expressed as mean grams/day/exposure period (standard deviation)

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$

Table 5.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Body Weight

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	Body Weight	148.1 (3.4)	155.3 (3.3)	165.7 (5.2)
700 ppm D4	Body Weight	142.8* (5.0)	153.7 (3.1)	163.2 (2.6)
PB (0.05%)	Body Weight	151.5 (4.7)	160.9* (3.1)	167.6 (4.5)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 10

Table 6.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Liver Weight and Liver-to-Body Weight Ratio

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	Liver Weight	5.3696 (0.3016)	5.2565 (0.3599)	5.4525 (0.5158)
700 ppm D4	Liver Weight	6.1072* (0.3312)	6.2308* (0.2835)	6.5538* (0.3225)
PB (0.05%)	Liver Weight	7.2848* (0.3555)	6.8968* (0.2266)	7.0217* (0.5546)
Control	Liver:Body Weight	0.0363 (0.0020)	0.0339 (0.0023)	0.0329 (0.0026)
700 ppm D4	Liver:Body Weight	0.0428* (0.0030)	0.0405* (0.0015)	0.0402* (0.0017)
PB (0.05%)	Liver:Body Weight	0.0481* (0.0018)	0.0429* (0.0013)	0.0419* (0.0031)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent mean liver weightexpressed in grams or the weight ratio (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 10

Table 7.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Thyroid Weight and Thyroid-to-Body Weight Ratio

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	Thyroid Weight	0.01334 (0.00212)	0.01309 (0.00164)	0.01123 (0.00106)
700 ppm D4	Thyroid Weight	0.01506 (0.00195)	0.01547* (0.00230)	0.01295* (0.00149)
PB (0.05%)	Thyroid Weight	0.01379 (0.00099)	0.01566* (0.00127)	0.01426* (0.00135)
Control	Thyroid:Body Weight	0.000090 (0.000015)	0.000085 (0.000011)	0.000068 (0.000006)
700 ppm D4	Thyroid:Body Weight	0.000106* (0.000015)	0.000101* (0.000015)	0.000080* (0.000009)
PB (0.05%)	Thyroid:Body Weight	0.000091 (0.000005)	0.000097* (0.000008)	0.000085* (0.000008)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent mean liver weightexpressed in grams or the weight ratio (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 9 - 10

Table 8.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rat Liver Hyperplasia

Treatment[1]	Liver Lobe	Measure[2]	Exposure Period[3]:		
			1	2	4
Control	Right	BrdU LI	7.12 (4.04)	8.98 (3.96)	13.93 (6.01)
700 ppm D4	Right	BrdU LI	22.32* (12.10)	10.96 (4.54)	8.69 (2.70)
PB (0.05%)	Right	BrdU LI	32.09* (7.40)	5.83 (1.65)	5.23* (2.47)
Control	Median	BrdU LI	8.57 (5.35)	12.66 (4.87)	15.62 (6.56)
700 ppm D4	Median	BrdU LI	22.58* (9.11)	14.92 (6.23)	9.86* (3.63)
PB (0.05%)	Median	BrdU LI	30.94* (7.96)	8.45 (2.33)	7.21* (2.23)
Control	Left	BrdU LI	5.09 (2.46)	8.05 (2.67)	14.38 (5.56)
700 ppm D4	Left	BrdU LI	16.89* (9.10)	11.61 (5.04)	8.58 (2.78)
PB (0.05%)	Left	BrdU LI	27.02* (6.65)	7.27 (2.80)	5.36* (2.85)
Control	Median	PCNA	3.44 (2.04)	3.30 (1.92)	5.31 (2.53)
700 ppm D4	Median	PCNA	13.20* (4.87)	7.33* (3.01)	5.56 (2.78)
PB (0.05%)	Median	PCNA	13.87* (4.11)	6.07* (2.58)	3.15* (1.28)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent the mean percent labeling indicies (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 10

Table 9.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rat Liver Hypertrophy

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	Hypertrophy	890 (23.2)	1039 (35.1)	1116 (104.4)
700 ppm D4	Hypertrophy	812* (58.9)	878* (58.9)	994* (80.6)
PB (0.05%)	Hypertrophy	726* (21.3)	842* (62.4)	830* (43.0)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent mean number of nuclei per fixed microscopic field (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 10

Table 10.

Phase II: Effect of D4 and PB Exposure of Female Fischer 344 Rat Thyroid Hyperplasia

Treatment[1]	Measure[2]	Exposure Period[3]:		
		1	2	4
Control	BrdU LI	1.19 (0.49)	1.39 (0.60)	1.39 (0.53)
700 ppm D4	BrdU LI	8.37* (5.11)	7.38* (4.44)	1.82 (0.80)
PB (0.05%)	BrdU LI	4.76* (1.94)	17.85* (6.64)	7.48* (4.10)

[1]Control (air) and D4 vapor was administered by whole-body inhalation (6 hours/day). PB was administered as a drinking water additive.

[2]Values represent mean Labeling index (standard deviation).

[3]1 = 1 five-day period (days 1-6); 2 = 2 five-day periods (days 1-13); 4 = 4 five-day periods (days 1 - 27)

*Significantly different from control at $p<0.05$; N = 10

Table 11.

Phase III: Clinical Observations Summary

Clinical Observation[1]	Incidence[2]							
	Control	7 ppm D4	30 ppm D4	70 ppm D4	150 ppm D4	300 ppm D4	700 ppm D4	0.05% PB
Normal	10 / 10	9 / 10	9 / 10	10 / 10	10 / 10	9 / 10	7 / 10	5 / 10
Urine Staining, perineal region	0 / 10	1 / 10	1 / 10	0 / 10	0 / 10	1 / 10	3 / 10	0 / 10
Porphyrin Staining, eye(s)	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	5 / 10

[1]Exposures to D4 were conducted for 6 hours per day for five consecutive days. Phenobarbital (PB) was administered as a drinking water additive for the five-day exposure period.

[2]Incidence is expressed as " the number of animals expressing the finding at least once during the study period / total number of animals in the treatment group"

Table 12.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Body Weight, Food and Water Consumption

Treatment[1]	Food Consumption[2]	Water Consumption[2]	Terminal Body Weight[3]
0 ppm D4	15.4 (1.0)	16.2 (0.7)	163.8 (3.9)
7 ppm D4	15.7 (1.1)	15.4 (2.1)	163.8 (2.8)
30 ppm D4	16.0 (1.1)	16.4 (1.2)	163.7 (4.3)
70 ppm D4	15.9 (1.3)	16.5 (1.5)	165.1 (3.1)
150 ppm D4	16.3 (1.1)	17.2 (1.5)	167.3 (4.4)
300 ppm D4	16.1 (1.2)	16.0 (1.9)	164.8 (3.9)
700 ppm D4	14.7 (0.8)	14.1* (1.1)	157.8* (3.2)
PB (0.05%)	17.7* (1.2)	20.2* (2.0)	168.4* (4.0)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean consumption expressed in grams/day (standard deviation).

[3]Values represent mean terminal body weight expressed as grams (standard deviation).

*Significantly different from control at $p<0.05$; N = 10

Table 13.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Liver Weight

Treatment[1]	Terminal Body Weight[2]	Liver Weight[3]	Liver:Body Weight
0 ppm D4	163.8 (3.9)	5.86 (0.323)	0.0358 (0.0014)
7 ppm D4	163.8 (2.8)	5.91 (0.475)	0.0361 (0.0025)
30 ppm D4	163.7 (4.3)	6.18 (0.512)	0.0377 (0.0023)
70 ppm D4	165.1 (3.1)	6.27 (0.277)	0.0380* (0.0015)
150 ppm D4	167.3 (4.4)	6.47* (0.294)	0.0387* (0.0013)
300 ppm D4	164.8 (3.9)	6.61* (0.249)	0.0401* (0.0013)
700 ppm D4	157.8* (3.2)	6.64* (0.283)	0.0421* (0.0016)
PB (0.05%)	168.4* (4.0)	7.56* (0.240)	0.0449* (0.0012)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]Values represent mean liver weight expressed as grams (standard deviation).

*Significantly different from control at $p<0.05$; N = 10

Table 14.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Thyroid Weight

Treatment[1]	Terminal Body Weight[2]	Thyroid Weight[3]	Thyroid:Body Weight[4]
0 ppm D4	163.8 (3.9)	0.0133 (0.0015)	0.081 (0.009)
7 ppm D4	163.8 (2.8)	0.0128 (0.0016)	0.078 (0.010)
30 ppm D4	163.7 (4.3)	0.0138 (0.0007)	0.084 (0.005)
70 ppm D4	165.1 (3.1)	0.0130 (0.0028)	0.079 (0.017)
150 ppm D4	167.3 (4.4)	0.0136 (0.0016)	0.082 (0.011)
300 ppm D4	164.8 (3.9)	0.0133 (0.0019)	0.081 (0.012)
700 ppm D4	157.8* (3.2)	0.0132 (0.0014)	0.084 (0.008)
PB (0.05%)	168.4 (4.0)	0.0149 (0.0026)	0.089 (0.016)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]Values represent mean thyroid weight expressed as grams (standard deviation).

[4]Values represent mean x 1000 (standard deviation x 1000)

*Significantly different from control at $p<0.05$; N = 10

Table 15.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Liver Hyperplasia

Treatment[1]	Liver Weight[2]	Liver BrdU LI[3]	Liver PCNA LI[3]
0 ppm D4	5.86 (0.323)	6.39 (1.59)	2.49 (1.14)
7 ppm D4	5.91 (0.475)	8.41 (3.32)	5.14* (2.57)
30 ppm D4	6.18 (0.512)	9.80 (4.54)	3.58 (1.54)
70 ppm D4	6.27 (0.277)	13.48* (4.94)	3.89* (1.45)
150 ppm D4	6.47* (0.294)	17.92* (4.63)	7.79* (2.62)
300 ppm D4	6.61* (0.249)	21.95* (7.22)	9.79* (2.94)
700 ppm D4	6.64* (0.283)	23.45* (9.55)	9.17* (3.08)
PB (0.05%)	7.56* (0.240)	27.66* (3.80)	17.06* (3.94)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]Values represent mean Labeling Index (standard deviation).

*Significantly different from control at $p<0.05$; N = 10

Table 16.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Liver Hypertrophy

Treatment[1]	Liver Weight[2]	Nuclei per Field[3]
0 ppm D4	5.86 (0.323)	1240 (125)
7 ppm D4	5.91 (0.475)	1313 (187)
30 ppm D4	6.18 (0.512)	1278 (83)
70 ppm D4	6.27 (0.277)	1297 (87)
150 ppm D4	6.47* (0.294)	1327 (125)
300 ppm D4	6.61* (0.249)	1318 (100)
700 ppm D4	6.64* (0.283)	1300 (130)
PB (0.05%)	7.56* (0.240)	1207 (150)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]Values represent mean number of nuclei per fixed microscopic field (standard deviation).

*Significantly different from control at $p<0.05$; N = 10

Table 17.

Phase III: Effect of D4 and PB Exposure of Female Fischer 344 Rats on Thyroid Hyperplasia

Treatment[1]	Thyroid Weight[2]	BrdU LI[3]
0 ppm D4	0.0133 (0.0015)	1.61 (1.16)
7 ppm D4	0.0128 (0.0016)	2.21 (1.74)
30 ppm D4	0.0138 (0.0007)	1.67 (1.51)
70 ppm D4	0.0130 (0.0028)	3.32 (3.20)
150 ppm D4	0.0136 (0.0016)	2.83 (2.48)
300 ppm D4	0.0133 (0.0019)	2.19 (2.38)
700 ppm D4	0.0132 (0.0014)	1.88 (1.11)
PB (0.05%)	0.0149 (0.0026)	2.42 (1.32)

[1]D4 was administered by whole-body vapor inhalation (6 hours/day for 5 days). PB was administered as a drinking water additive.

[2]Values represent mean terminal body weight expressed in grams (standard deviation).

[3]Values represent mean BrdU Labeling Index (standard deviation).

*Significantly different from control at $p<0.05$; N = 9-10

APPENDIX A

Contributing Scientist Report: Statistics

Effects of Octamethylcyclotetrasiloxane (D4) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28-Day Inhalation Study

MEMORANDUM
To: Paul Jean
From: Trevor Newhook
Study: 8491

This document contains the statistical analysis of the data from Study 8491, which I carried out on 28th June, 2002.

Signature Trevor Newhook Date 11-21-2002

Statistical Methods

All data analysis was carried out using SAS® version 8.2. In all comparisons, the family wise error rate was held at 5% ($\alpha = 0.05$).

In phase I, the organ cellular proliferation data and body and organ weight data were analyzed using a one-way analysis of variance. Because there were only two treatment groups, a statistically significant global F-test indicated a significant difference between the two treatment groups and no comparison of means was required. The Wilcoxon test was used in any situations where the data was not normally distributed or the variances were not equal among the groups, since there were only two groups.

In phase II, the organ cellular proliferation data were first evaluated with a three way analysis of variance (main effects: chamber, treatment, and day) to determine if there were statistically significant differences within groups due to the use of more than one chamber per exposure level. In those cases where chamber was a significant effect, individual contrasts were constructed to compare the mean response within each of the two chambers in each treatment group. The cellular proliferation data, organ and body weight data and food and water consumption data were then analyzed using a two-way analysis of variance (main effects: treatment and day). Where significant day*treatment interactions existed, the final analysis was a separate one-way analysis of variance (main effect: treatment) for each day. When the global F-test indicated a significant treatment effect, Dunnett's test for multiple comparisons against the control was used to compare the mean response in animals treated with either D_4 or phenobarbital to the mean response in control animals. This method was used instead of Tukey's test for all possible comparisons because there was no interest in the comparison of the mean response in animals treated with D_4 to the mean response in animals treated with the intra-assay positive control (phenobarbital) and because Dunnett's test is more powerful for determining a treatment effect under this design.

In phase III, organ cellular proliferation data, organ and body weight data and food and water consumption data from the control animals and those treated with D_4 were analyzed with a one-way analysis of variance (main effect: dose). When the global F-test indicated a significant treatment effect, Dunnett's test for multiple comparisons against the control was used to compare the mean response in animals in each dose group against the mean response in animals in the control group. This test was used instead of sequential t-tests because it uses the pooled estimate of the variance, which is the best estimate and is a critical factor in controlling Type I error, and because this procedure properly accounts for the correlation among tests due to the fact that they are all made against a common control group.

RESULTS

Phase I:

Phase I of this study was designed to establish the technical skills and methodologies associated with BrdU and PCNA tissue handling, processing, and immunohistochemical evaluations. Daily administration of Wyeth 14,643 for four consecutive days resulted in a statistically significant increase (approximately 37%) in liver weight and liver-to-body weight ratio without significantly affecting body weight. The effect on liver weight accompanied a statistically significant increase in the LI for PCNA (4.2-fold) and BrdU (7.5-fold)

Phase II:

Phase II was conducted to evaluate the effect of exposure to 700 ppm D4 vapor on cell proliferation (liver and thyroid) and hypertrophy (liver) as a function of exposure duration. In this phase, female Fischer 344 rats were exposed to 695, 696 and 700 ppm D4 vapor for 1, 2, or 4 five-day exposure periods, respectively. Note that each five-day exposure period was separated by a two-day non-exposure period. Control (air exposed) and PB treated rats were placed into inhalation chambers, but not exposed to D4 vapor, to mimic the exposure routine of the D4 exposed animals. Body weight and consumption of food and water were monitored weekly. D4 exposure had no effect on food and water consumption over any of the exposure periods. Exposure to D4 resulted in a statistically significant 4% decrease in body weight as observed after 1 five-day exposure period. In contrast, PB-treated rats demonstrated statistically significant increases in food and water consumption for each of the 1, 2, and 4 five-day treatment periods and a statistically significant 4% increase in body weight at the end of the second five-day treatment period.

In Phase II exposure to D4 resulted in increased liver weight and liver-to-body weight ratios following 1, 2, and 4 five-day exposure periods. The increases in liver weight (liver-to-body weight ratio) were 14(18), 19(19) and 20(22) percent greater than control after 1, 2 and 4 five-day exposure periods, respectively. Similarly, exposure to D4 resulted in increased thyroid and thyroid-to-body weight ratios following 1, 2, and 4 five-day exposure periods. The increases were 13(18), 18(19) and 15(18) percent greater than control thyroid weight (thyroid-to-body weight) after 1, 2, and 4 five-day exposure periods, respectively. These increases were statistically significant except for thyroid absolute weight at the end of the first five-day treatment period.

Accompanying the D4-induced increase in liver weight was a statistical increase in hyperplasia as demonstrated by increases in the labeling indices for both BrdU and PCNA analysis. The LI for BrdU was increased approximately 3-fold after 1 five-day period of exposure in each of the three lobes evaluated. After the second five-day period of exposure to D4 the LI for BrdU was not statistically different from control in any lobe and after the fourth five-day period of exposure to D4 the LI for BrdU was statistically significantly lower in the medium lobe. The PCNA analysis of the median lobe gave statistically significant results with LI increases of 3.8- and 2.2-fold after 1 and 2 five-day exposure periods, respectively. The PCNA LI was statistically significantly lower than the control after 4 five-day exposure periods.

Exposure to PB in Phase II resulted in a statistically significantly increased liver and liver-to-body weight ratios following 1, 2, and 4 five-day exposure periods. The increases were 36(33), 31(27) and 29(27) percent greater than control liver weight (liver-to-body weight ratio) after 1, 2 and 4 five-day exposure periods, respectively. Thyroid and thyroid-to-body weight ratios were statistically significantly increased

after 2 and 4 five-day exposure periods. The increases were 20(15) and 31(29) percent greater than control values after 2, and 4 five-day exposure periods, respectively.

Accompanying the PB-induced increase in liver weight was an increase in hyperplasia as demonstrated by increased labeling indices for both BrdU and PCNA analysis. In each of the three lobes evaluated, the LI for BrdU was statistically significantly increased approximately 3-fold after 1 five-day period of exposure. After 2 five-day periods of exposure to PB the LI for BrdU was not different from control and after 4 five-day PB exposure periods the LI was statistically significantly lower in each lobe compared to the controls. The PCNA analysis of the median lobe gave similar results with LI increases after 1 and 2 five-day exposure periods of 4-fold and 1.8-fold, respectively. After 4 five-day periods of exposure to PB the PCNA LI was 40% lower than control.

Liver hypertrophy, as measured by a reduction in the number of nuclei within a fixed microscopic field size, was demonstrated for both D4 and PB exposed animals. The mean number of nuclei per field was reduced by 9, 15, and 11% in rats following 1, 2, and 4 five-day D4 exposure periods, respectively. Similarly, the mean number of nuclei per field was reduced by 18, 18, and 26% in rats following 1, 2, and 4 five-day PB exposure periods, respectively. Each of these decreases was statistically significant except for the 18% decrease following the first five-day PB exposure.

Thyroid cell hyperplasia, as measured by BrdU LI, was increased by D4 and PB treatments. Exposure to D4 for 1 and 2 five-day exposure periods was shown to cause a statistically significant 7- and 5-fold increase in BrdU LI, respectively. Exposure to PB for 1, 2, and 4 five-day exposure periods was shown to cause a statistically significant 4-, 13-, and 5- fold increase in BrdU LI, respectively.

Phase III:

Phase III was conducted to determine the dose-responsiveness of hyperplasia (liver and thyroid) and hypertrophy (liver) after the first five-day vapor inhalation exposure to D4. This exposure duration represents the optimal duration for assessing liver hyperplasia as demonstrated in phase II. In this phase female Fischer 344 rats were exposed to 0, 7, 30, 70, 150, 300, and 700 ppm D4 vapor for 1 five-day exposure period. One additional group of rats were included as a positive control receiving PB (drinking water) and not exposed to D4 vapor. These animals were placed into inhalation chambers to mimic treatment conditions of the D4-treated animals. Body weight and consumption of food and water were monitored. Exposure to D4 had no statistically significant affect on food and water consumption with exception to a 13% decrease in water consumption at the 700 ppm exposure level. Body weight was similarly unaffected expect for a statistically significant 13% decrease at the 700 ppm exposure level. Food and water consumption and body weight were significantly increased by PB-treatment by 15, 25 and 3%, respectively.

Exposure to D4 in Phase III resulted in statistically significant increases in liver weight and liver-to-body weight ratios at exposure concentrations greater than 70 ppm and 30 ppm, respectively. The increases in liver weight (liver-to-body weight ratio) were 7(6), 10(8), 13(12), and 13(18) percent greater than control for exposure concentrations of 70, 150, 300 and 700 ppm D4, respectively. The mean thyroid and thyroid-to-body weight ratios were not significantly different compared to the control at any dose levels.

Accompanying the D4-induced increase in liver weight was a dose-responsive increase in hyperplasia as demonstrated by statistically significant increases in the labeling indices for both BrdU and PCNA analysis. This study phase utilized only the median lobe of the liver, a decision based on the study results

from phase II that demonstrated that hyperplasia was not lobe specific. A statistically significant increase in LI for BrdU was observed for exposure to 70 (2-fold), 150 (3-fold), 300 (3-fold) and 700 (4-fold) ppm D4. Similarly, statistically significant increases in LI for PCNA were observed exposure to 70 (2-fold), 150 (3-fold), 300 (4-fold) and 700 ppm D4 (3.7-fold). A statistically significant increase in PCNA LI was also obtained for rats in the 7 ppm D4 exposure group (2.1-fold increase) but not for rats exposed to 30 ppm D4 (1.4-fold increase).

Exposure to PB in Phase III resulted in statistically significant increased liver and liver-to-body weight ratios following 1 five-day exposure period. The increases were 29%, and 25% greater than control values for liver weight and liver-to-body weight ratio, respectively. Thyroid and thyroid-to-body weight ratios were increased by 12% and 9% but these increases were not statistically significant.

Accompanying the PB-induced increase in liver weight was a statistically significant increase in hyperplasia as demonstrated by increased labeling indices for both BrdU and PCNA analysis. The LI for BrdU was increased 4.8-fold and the LI for PCNA was increased 6.9-fold after the 1 five-day period of exposure.

Liver hypertrophy, as measured by a reduction in the number of nuclei within a fixed microscopic field size, was not observed for D4 or PB exposed animals in contrast to the results obtained in phase II. The mean number of nuclei per field for the D4 and PB treated animals was not statistically different from the control values at any of the exposure concentration levels.

The thyroid cell hyperplasia as measured by BrdU LI, was not evident following exposure to either D4 or PB in this phase. The LI for BrdU in the D4 and PB treated animals was not statistically different from the control values at any of the exposure concentration levels.

APPENDIX B

Inhalation Methods Summary Report for Study 8491

Effects of Octamethylcyclotetrasiloxane (D4) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28-Day Inhalation Study

SUMMARY

Phase II:
Female Fischer 344 rats were exposed to octamethylcyclotetrasiloxane (D4) vapor for 1, 2 or 4 five-day exposure periods. Each successive five-day exposure period was separated by a two-day non-exposure period. Daily exposures consisted of a 6 hr exposure to a target vapor concentration of 700 ppm D4 sandwiched between T_{99} periods of 20 minutes each. Mean exposure concentrations (standard deviation) for animals in each of the three exposure subgroups were 698 (12.3), 697 (12.3), and 700 (11.5) ppm D4 for 1, 2, and 4 five-day exposure periods, respectively. Animals in the control and phenobarbital (PB) treatment groups were placed in inhalation chambers daily for the same duration as the D4 exposed animals but they were not exposed to D4 vapor. The range of mean daily chamber temperatures and humidity for all exposure chambers were 20-24.5°C and 34 – 45%, respectively.

Phase III:
Female Fischer 344 rats were exposed to D4 vapor for 1 five-day exposure period. Daily exposures consisted of a 6 hr exposure to target vapor concentration (0, 7, 30, 70, 150, 300, or 700 ppm D4) sandwiched between T_{99} periods of 20 minutes each. Mean exposure concentrations (standard deviation) for animals in the 7, 30,70, 150, 300, and 700 ppm D4 target exposure groups were 7(0), 29(1), 70(3), 150(5), 300(8) and 701(12) ppm D4, respectively. Animals in the 0 ppm D4 and PB treatment groups were placed in inhalation chambers daily for the same duration as the D4 exposed animals but they were not exposed to D4 vapor. The range of mean daily chamber temperatures and humidity for all exposure chambers were 20.8-24.3°C and 35.9 – 46.7%, respectively.

MATERIALS AND METHODS

A. Inhalation Exposure Methods

Exposures were conducted in 450-liter Rochester style stainless steel and glass whole body chambers (Figure 1) operated under dynamic conditions (chamber pressure: ~ -0.6 in. H_20). The air supplied to the inlet of the chambers was room air that had been passed through activated carbon and HEPA filters. Chamber airflow rates were measured using sharp-edged orifice plates (Doebelin, 1983) and differential pressure transducers (Validyne model DP 851C-P10) equipped with digital displays. The pressure transducers were calibrated prior to study initiation using an NIST traceable Modus digital manometer (Model MA2-0021). Chamber airflow was maintained at 12-15 air changes per hour resulting in a T_{99} of 20 minutes (Silver, 1946). Each chamber was leak tested at normal operating parameters prior to the start of the study. As an extra precaution, chambers were operated under slightly negative pressures (~ -0.6" H_2O) during exposures. Chamber temperature and relative humidity were monitored using Omega® Engineering, Inc. sensors (Model HX11-C) equipped with digital displays. Each sensor was calibrated prior to start of the study using an NIST traceable thermal coupler (Omega® HH11 for temperature) and NIST traceable Hygrometer (Fisher Scientific Digital Humidity/ Temperature meter for humidity). Airflow, temperature, and humidity were monitored continuously and manually recorded every thirty minutes during each exposure period. Prior to the start of each phase, chamber test article homogeneity was evaluated for all chambers in which test article was generated. During this evaluation, test article was introduced into each exposure chamber at or near the appropriate target concentration. The chamber atmosphere was then sampled three times each at six different locations from within the chamber. The average area count response for each location was calculated and compared to results from the reference location. A difference between the reference and other sample locations of less than or equal to ten percent was considered acceptable. Homogeneity evaluations for all chambers were within acceptable limits.

Octamethylcyclotetrasiloxane vaporization was conducted using glass J-tubes (Miller *et al.*, 1980) containing 6 mm soda lime glass beads. J-tubes were wrapped with heat tape and insulation and

operated at a temperature of between 70-80°C to promote efficient vaporization of D4 (Figure 2). Fluid Metering, Inc. (FMI) pumps were used to meter D4 at a constant flow rate from glass reservoirs into the J-tubes. Grade D breathable air, supplied from a NASH compressor, was passed as the carrier gas through the J-tubes at a controlled rate. The air/D4 vapor exiting the J-tube was then introduced into the inlet port at the top of the chambers for dilution into the make-up air stream.

B. Test Article Monitoring

1. Nominal:

The amount of test article used during each exposure period was determined by measuring the difference between the pre- and post-exposure weight of the test article reservoirs. The vapor generation time (one T_{99} plus the six hour exposure period), test article consumption, and total volume of air through the chambers were used to calculate nominal concentration values (Silver, 1946).

2. Measured:

A Varian® 3400 gas chromatograph (G.C. conditions are presented in Table 1) operated by a Varian Star® Workstation was used for analytical determination of chamber concentrations (Figure 3). The instrument was equipped with a flame ionization detector (FID), a Valco® 8 port (phase II) or Valco® 12 port (phase III) stream selector valve, and a heated Valco® injector valve. Five point calibrations were performed prior to the start of the study by preparing samples in Tedlar® gas sampling bags. Two bag standards of known concentration were prepared for each of five calibration levels by injecting a known volume of D4 into a measured volume of air. A heat gun was then used to completely vaporize the D4 inside the bag. Each bag was sampled twice for a total of twenty sample points. The Varian Star® software (Version 4.5), was then used to generate a calibration curve (origin was not used as a sample point) for each G.C. method. Multiple calibration curves were used to span the large range of exposure concentrations. Before each exposure a bag standard was prepared for each calibration curve and analyzed using the appropriate method for verification of the performance of the G.C. During the daily G.C. verification, the bag standards were attached to the sample lines at the chambers. If the response for the bag standard was not within ten percent of the expected area count response, a second standard was prepared. If the result was still not within acceptable limits, the GC was re-calibrated following the exposure and the daily results recalculated. During conduct of an exposure, each chamber was analyzed a minimum of once an hour for the duration of the exposure period.

3. Sampling System:

Samples of the chamber test atmosphere were drawn from the exposure chambers through ¼" O.D. Teflon® tubing and a Valco® stream selector valve using Thomas vacuum pumps (Model 917CA18). One pump was used for the sample line and the second pump was used to for continuous purge of the other sample lines. The samples were drawn under negative pressure through a 229 µL stainless steel sample loop and injected onto the column using an air actuated Valco® injector valve. Prior to initiation of the study, sample line flow rates and sample line loss were measured to confirm the integrity of the air sampling system. Flow rates and sample line loss rates were within acceptable tolerances.

C. Exposure Procedures

Before each exposure, animals were transferred from their housing caging into exposure caging (individual compartments approximately 6" W x 6" H x 7" L). Animal positions in these exposure

cages were rotated front to back, and top to bottom, on a daily basis to eliminate any bias related to potential differences in concentrations among positions within the chamber. Animals were caged individually in both the housing and exposure caging. To avoid confusion during transfer, the exposure cages and the chambers were labeled with color-coded tags. Following each exposure period, the animals were returned to their housing caging. Food and water were not available to the animals during the exposure periods.

RESULTS

Phase II:

Daily mean chamber temperature and humidity for all of the exposure chambers combined ranged between 20.0 - 24.5 °C and 34.0 - 45.1% relative humidity, respectively. The daily mean individual chamber temperature and humidity values are summarized in table 2 (this appendix). The mean chamber D4 vapor concentrations for the D4 exposure group were 698(12.3), 697(12.3), 700 (11.5) ppm D4 for 1, 2 and 4 five-day exposure periods. The individual chamber daily mean exposure concentrations are summarized in table 3 (this appendix).

Phase III:

Daily mean chamber temperature and humidity for all of the exposure chambers ranged between 20.8 - 24.3°C and 35.9 - 46.7% relative humidity, respectively. The daily mean individual chamber temperature and humidity values are summarized in table 4 (this appendix). The mean chamber D4 vapor concentrations for the various D4 exposure groups were 7(0), 29(1), 70(3), 150(5), 300(8), and 701(12) ppm D4 for the 7, 30, 70, 150, 300, and 700 ppm D4 target concentration exposure groups, respectively. The individual chamber daily mean exposure concentrations are summarized in table 5 (this appendix).

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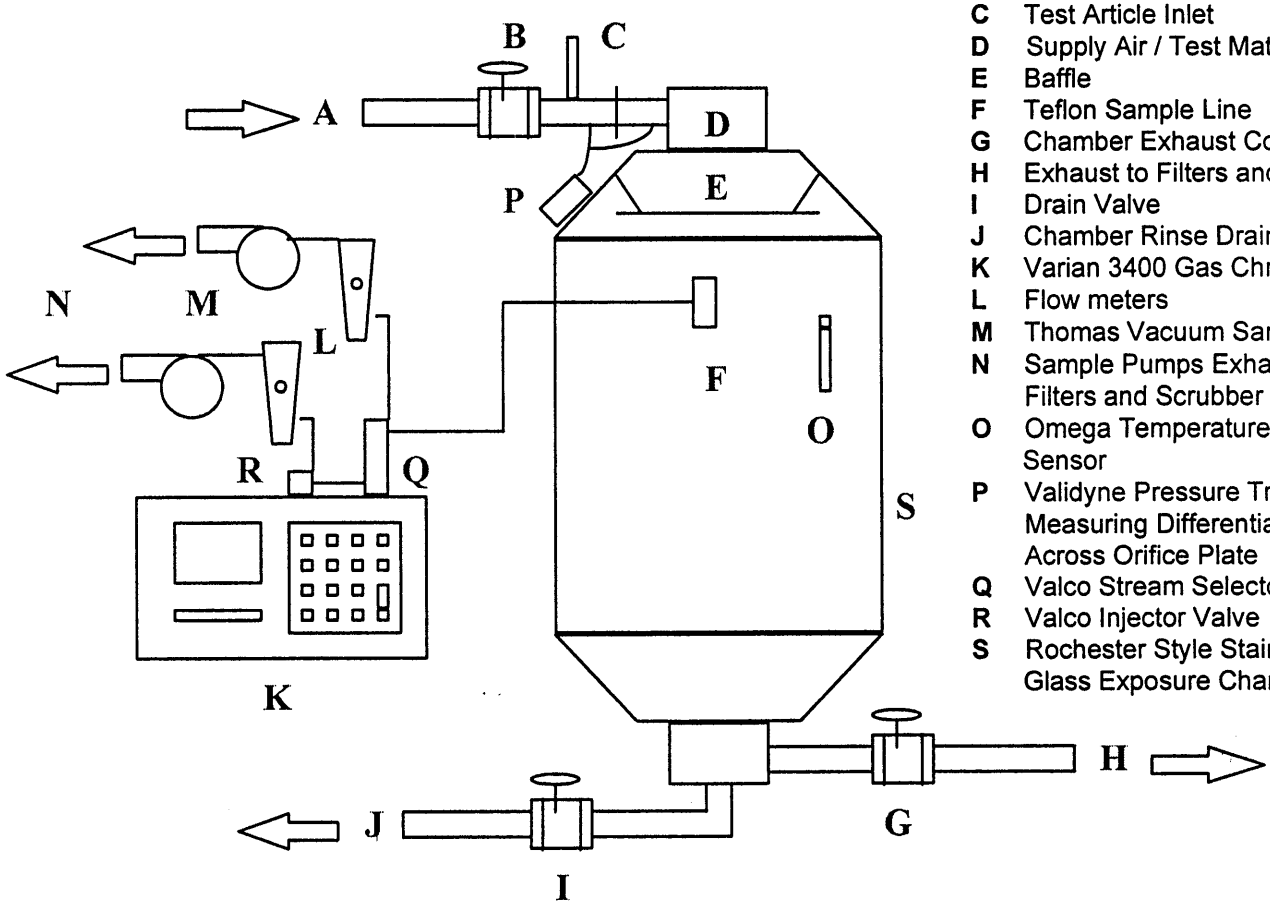
Appendix B

Figure 1

Diagram of Exposure Chamber and Sampling System



A Filtered Chamber Supply Air
B Supply Air Control Valve
C Test Article Inlet
D Supply Air / Test Material Mixing
E Baffle
F Teflon Sample Line
G Chamber Exhaust Control Valve
H Exhaust to Filters and Scrubber
I Drain Valve
J Chamber Rinse Drain
K Varian 3400 Gas Chromatagraph
L Flow meters
M Thomas Vacuum Sampling Pumps
N Sample Pumps Exhausted to Filters and Scrubber
O Omega Temperature / Humidity Sensor
P Validyne Pressure Transducer Measuring Differential Pressure Across Orifice Plate
Q Valco Stream Selector Valve
R Valco Injector Valve
S Rochester Style Stainless Steel and Glass Exposure Chamber (450 L)

Appendix B

Figure 2

Diagram of Vapor Generation System

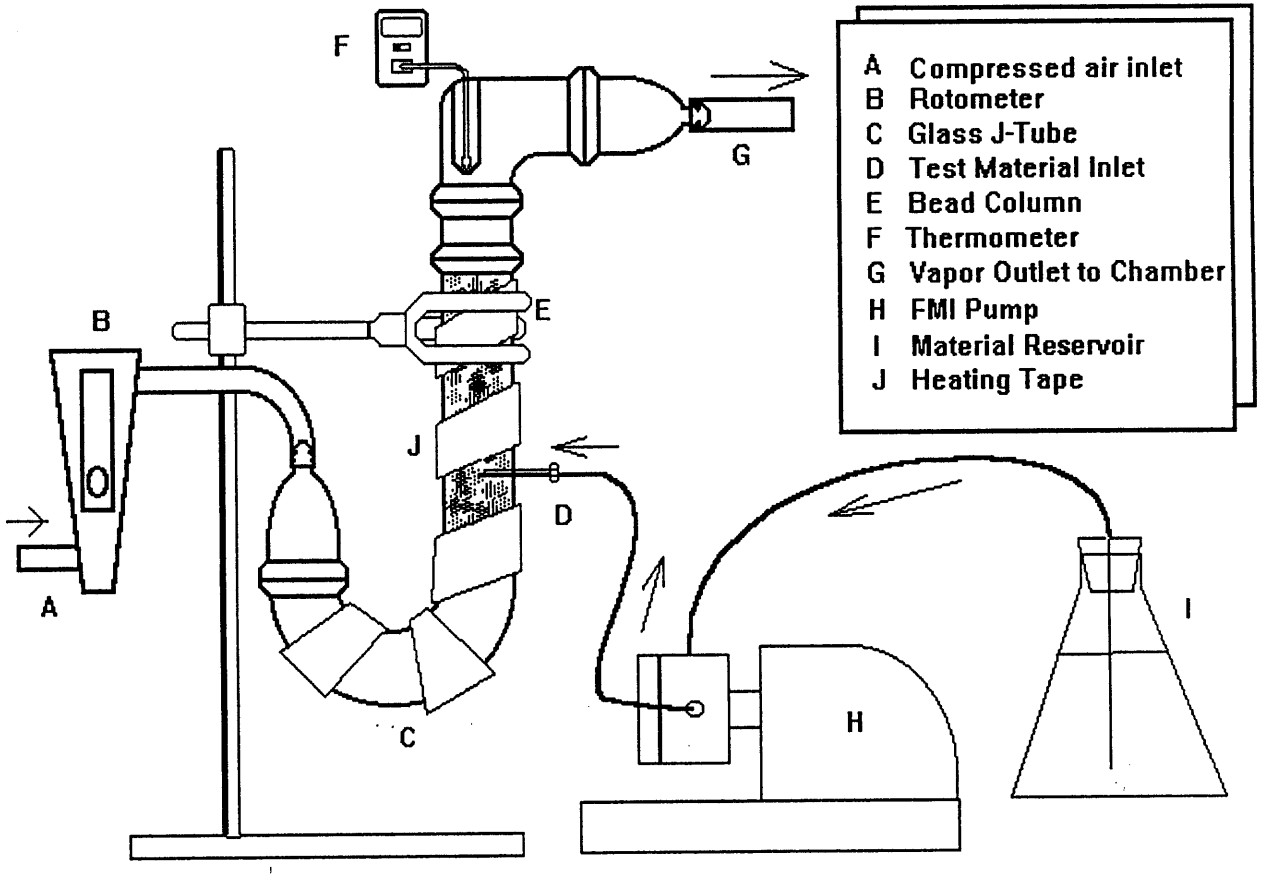
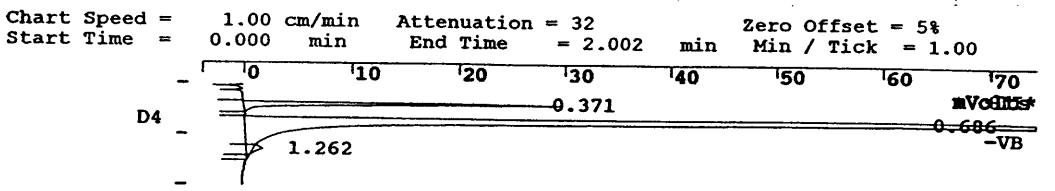


Figure 3

Typical Gas Chromatography Chromatogram



Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

Peak No.	Peak Name	Result (PPM)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0	0.371	0.000	8499	BB	2.5	
2	D4	30	0.686	0.002	203814	BB	2.6	
3		0	1.262	0.000	476	TS	0.0	
	Totals:	30		0.002	212789			

Total Unidentified Counts : 8976 counts

Detected Peaks: 3 Rejected Peaks: 0 Identified Peaks: 1

Amount Standard: N/A Multiplier: 1.000000 Divisor: 1.000000

Baseline Offset: -14 microVolts

Noise (used): 170 microVolts - monitored before this run

Stream: 10 Injection Number: 1 Sampling Time: 0.00 min

Table 1

Gas Chromatography Conditions for Octamethylcyclotetrasiloxane Analysis

Gas Chromatograph (GC)

Model:	Varian® 3400
Identification:	13440
Software:	Varian® Star version 4.5 for Windows® 95

GC Injector

Initial temperature:	225 degrees C (Isothermal)

GC Column

Model:	J & W DB-5
Serial number:	1277624a
Length:	15 meter
Film thickness:	1.5 µm
Carrier gas:	Helium
Temperature:	130 degrees C (Isothermal)
Hold time:	6 minutes

GC Detector

Type:	FID
Temperature:	250 degrees C

Flow Rates

Air:	300 ml/min.
Helium:	10 ml/min.
Helium + make-up:	30 ml/min.
Hydrogen:	30 ml/min.

Autosampler

Type:	Valco stream selector valve
Sample loop volume:	229 µL

Table 2

Chamber Temperature and Humidity Summary: Phase II

0 ppm D4 Exposure Group: Phase II							
Five-day Exposure Period	Study Day	Mean Daily Temperature (°C)			Mean Daily Humidity (%)		
		Chamber 1	Chamber 2	Mean	Chamber 1	Chamber 2	Mean
First	1	22.9	22.2	**22.6**	36.7	37.1	**36.9**
	2	22.8	22.5	**22.7**	36.8	34.8	**35.8**
	3	22.2	21.2	**21.7**	38.5	38.1	**38.3**
	4	21.1	20.7	**20.9**	39.9	37.1	**38.5**
	5	21.6	20.6	**21.1**	39.6	38.1	**38.9**
			Mean =	**21.8**		Mean =	**37.7**
			Std dev =	**0.8**		Std dev =	**1.3**
Second	8	21.7	20.6	**21.2**	36.6	35.1	**35.9**
	9	21.4	20.5	**21.0**	40.3	38.2	**39.3**
	10	22.1	21.2	**21.7**	41.9	38.1	**40.0**
	11	21.3	20.2	**20.8**	42.2	40.9	**41.6**
	12	21.5	20.8	**21.1**	42.2	39.0	**40.6**
			Mean[1] =	**21.2**		Mean[1] =	**38.6**
			Std dev[1] =	**0.3**		Std dev[1] =	**1.9**
Third	15	21.6	20.8	**21.2**	42.2	38.8	**40.5**
	16	21.2	20.1	**20.7**	42.6	38.6	**40.6**
	17	21.4	20.4	**20.9**	41.2	38.6	**39.9**
	18	21.2	20.0	**20.6**	42.0	40.0	**41.0**
	19	21.5	20.4	**21.0**	41.4	38.4	**39.9**
Fourth	22	21.5	20.2	**20.9**	38.9	38.5	**38.7**
	23	21.9	21.1	**21.5**	41.6	39.7	**40.7**
	24	21.5	20.5	**21.0**	43.7	41.9	**42.8**
	25	21.7	20.7	**21.2**	43.7	41.9	**42.8**
	26	21.1	20.1	**20.6**	45.1	43.4	**44.3**
			Mean[2] =	**21.3**		Mean[2] =	**39.8**
			Std dev[2]=	**0.7**		Std dev[2]=	**2.2**

[1]Mean and standard deviation of values for the entire period (1 and 2 five-day exposures)
[2]Mean and standard deviation of values for the entire period (1-4 five-day exposures)

Table 2

Chamber Temperature and Humidity Summary: Phase II
(Continued)

700 ppm D4 Exposure Group: Phase II							
Five-day Exposure Period	Study Day	Mean Daily Temperature (°C)			Mean Daily Humidity (%)		
		Chamber 1	Chamber 2	Mean	Chamber 1	Chamber 2	Mean
First	1	23.9	23.3	**23.6**	36.5	36.1	**36.3**
	2	23.7	23.4	**23.6**	37.0	36.2	**36.6**
	3	23.1	22.8	**23.0**	36.6	36.5	**36.6**
	4	21.9	22.5	**22.2**	38.6	37.5	**38.1**
	5	22.5	22.8	**22.7**	37.1	36.6	**36.9**
			Mean =	**23.0**		Mean =	**36.9**
			Std dev =	**0.6**		Std dev =	**0.7**
Second	8	22.6	22.6	**22.6**	36.0	36.1	**36.1**
	9	22.2	22.8	**22.5**	34.1	32.7	**33.4**
	10	22.0	21.9	**22.0**	35.9	36.5	**36.2**
	11	22.2	22.0	**22.1**	35.1	35.6	**35.4**
	12	21.5	21.9	**21.7**	37.9	36.0	**37.0**
			Mean[1] =	**22.6**		Mean[1] =	**36.2**
			Std dev[1] =	**0.6**		Std dev[1] =	**1.2**
Third	15	22.0	22.9	**22.5**	36.9	35.5	**36.2**
	16	21.9	22.3	**22.1**	36.4	35.2	**35.8**
	17	21.7	22.2	**22.0**	36.3	35.3	**35.8**
	18	21.9	22.1	**22.0**	36.7	36.3	**36.5**
	19	21.8	22.3	**22.1**	36.0	35.0	**35.5**
Fourth	22	21.9	22.2	**22.1**	34.7	34.0	**34.4**
	23	22.5	22.6	**22.6**	35.2	34.9	**35.1**
	24	22.3	22.5	**22.4**	37.3	36.7	**37.0**
	25	22.3	22.7	**22.5**	38.1	37.5	**37.8**
	26	21.7	21.9	**21.8**	38.6	38.3	**38.5**
			Mean[2] =	**22.4**		Mean[2] =	**36.2**
			Std dev[2]=	**0.5**		Std dev[2]=	**1.2**

[1]Mean and standard deviation of values for the entire period (1 and 2 five-day exposures)
[2]Mean and standard deviation of values for the entire period (1-4 five-day exposures)

Chamber Temperature and Humidity Summary: Phase II
(Continued)

Phenobarbital Exposure Group: Phase II							
Five-day Exposure Period	Study Day	Mean Daily Temperature (°C)			Mean Daily Humidity (%)		
		Chamber 1	Chamber 2	Mean	Chamber 1	Chamber 2	Mean
First	1	22.4	22.6	**22.5**	38.1	40.2	**39.2**
	2	22.4	23.0	**22.7**	37.6	38.8	**38.2**
	3	21.8	22.0	**21.9**	38.7	40.2	**39.5**
	4	21.0	21.5	**21.3**	39.4	41.4	**40.4**
	5	21.3	21.6	**21.5**	38.8	40.1	**39.5**
			Mean =	**22.0**		Mean =	**39.3**
			Std dev =	**0.6**		Std dev =	**0.8**
Second	8	21.3	21.4	**21.4**	37.3	37.4	**37.4**
	9	21.3	21.1	**21.2**	40.3	40.2	**40.3**
	10	21.7	21.9	**21.8**	42.0	42.5	**42.3**
	11	21.2	21.2	**21.2**	42.5	42.1	**42.3**
	12	21.6	20.5	**21.1**	41.8	44.7	**43.3**
			Mean[1] =	**21.6**		Mean[1] =	**40.2**
			Std dev[1] =	**0.6**		Std dev[1] =	**1.9**
Third	15	21.5	21.5	**21.5**	41.6	42.2	**41.9**
	16	20.9	21.1	**21.0**	42.4	42.0	**42.2**
	17	21.5	21.1	**21.3**	40.1	41.8	**41.0**
	18	21.1	21.3	**21.2**	40.5	40.2	**40.4**
	19	21.6	21.4	**21.5**	40.1	41.2	**40.7**
Fourth	22	21.3	21.4	**21.4**	38.2	38.9	**38.6**
	23	22.0	22.0	**22.0**	39.3	40.5	**39.9**
	24	21.7	21.8	**21.8**	41.3	42.2	**41.8**
	25	21.7	21.6	**21.7**	42.5	43.8	**43.2**
	26	21.1	21.3	**21.2**	44.0	44.2	**44.1**
			Mean[2] =	**21.5**		Mean[2] =	**40.8**
			Std dev[2]=	**0.5**		Std dev[2]=	**1.8**

[1]Mean and standard deviation of values for the entire period (1 and 2 five-day exposures)
[2]Mean and standard deviation of values for the entire period (1-4 five-day exposures)

Table 3

Chamber D4 vapor Concentration Summary: Phase II

700 ppm D4 Exposure Group: Phase II				
Five-day Exposure Period	Study Day	Mean Daily Chamer Concentrations (ppm D4)		
		Chamber 1	Chamber 2	Mean
First	1	715	673	**694**
	2	710	691	**701**
	3	702	719	**711**
	4	710	700	**705**
	5	689	668	**679**
			Mean =	**698**
			Std dev =	**12.3**
Second	8	687	659	**673**
	9	698	700	**699**
	10	701	695	**698**
	11	699	701	**700**
	12	708	711	**710**
			Mean[1] =	**697**
			Std dev[1] =	**12.3**
Third	15	707	706	**707**
	16	706	692	**699**
	17	679	708	**694**
	18	696	699	**698**
	19	730	722	**726**
Fourth	22	714	713	**714**
	23	692	701	**697**
	24	693	693	**693**
	25	711	700	**706**
	26	711	697	**704**
			Mean[2] =	**700**
			Std dev[2]=	**11.5**

[1]Mean and standard deviation of values for the entire period (1 and 2 five-day exposures)
[2]Mean and standard deviation of values for the entire period (1-4 five-day exposures)

Table 4

Chamber Temperature and Humidity Summary: Phase III

Exposure Concentration	Study Day	Mean Daily Temperature (°C)			Mean Daily Humidity (%)		
		Subgroup A[1]	Subgroup B[1]	Mean	Subgroup A[1]	Subgroup B[1]	Mean
0 ppm D4	1	22.4	21.5	**22.0**	43.1	46.5	**44.8**
	2	21.0	21.2	**21.1**	44.0	46.7	**45.4**
	3	21.9	21.8	**21.9**	44.0	44.8	**44.4**
	4	21.0	21.2	**21.1**	44.9	45.5	**45.2**
	5	21.5	21.7	**21.6**	45.0	44.8	**44.9**
			Mean =	**21.5**		Mean =	**44.9**
			Std dev =	**0.4**		Std dev =	**0.4**
7 ppm D4	1	23.2	22.6	**22.9**	41.4	43.5	**42.5**
	2	21.8	21.6	**21.7**	43.6	45.8	**44.7**
	3	23.1	22.7	**22.9**	42.0	42.2	**42.1**
	4	21.6	21.7	**21.7**	44.4	44.7	**44.6**
	5	22.3	22.6	**22.5**	43.1	42.6	**42.9**
			Mean =	**22.3**		Mean =	**43.3**
			Std dev =	**0.6**		Std dev =	**1.2**
30 ppm D4	1	22.6	22.0	**22.3**	41.4	44.7	**43.1**
	2	21.7	21.7	**21.7**	42.9	44.5	**43.7**
	3	22.4	22.4	**22.4**	42.2	43.0	**42.6**
	4	21.6	21.8	**21.7**	43.1	44.2	**43.7**
	5	21.8	22.2	**22.0**	43.5	43.5	**43.5**
			Mean =	**22.0**		Mean =	**43.3**
			Std dev =	**0.3**		Std dev =	**0.5**
70 ppm D4	1	23.1	22.5	**22.8**	41.8	44.3	**43.1**
	2	22.1	22.4	**22.3**	43.1	43.6	**43.4**
	3	22.7	22.9	**22.8**	42.7	42.7	**42.7**
	4	21.9	22.2	**22.1**	43.9	43.3	**43.6**
	5	22.4	22.7	**22.6**	43.9	43.0	**43.5**
			Mean =	**22.5**		Mean =	**43.2**
			Std dev =	**0.3**		Std dev =	**0.4**

Table 4

Chamber Temperature and Humidity Summary: Phase III
(Continued)

Exposure Concentration	Study Day	Mean Daily Temperature (°C)			Mean Daily Humidity (%)		
		Subgroup A[1]	Subgroup B[1]	Mean	Subgroup A[1]	Subgroup B[1]	Mean
150 ppm D4	1	22.6	22.1	**22.4**	41.1	43.3	**42.2**
	2	21.6	21.8	**21.7**	42.7	43.4	**43.1**
	3	22.3	22.3	**22.3**	41.5	42.2	**41.9**
	4	21.6	21.6	**21.6**	42.5	44.6	**43.6**
	5	21.9	22.2	**22.1**	42.7	42.5	**42.6**
			Mean =	**22.0**		Mean =	**42.7**
			Std dev =	**0.3**		Std dev =	**0.7**
300 ppm D4	1	23.2	22.4	**22.8**	35.9	41.8	**38.9**
	2	22.1	21.8	**22.0**	40.2	42.7	**41.5**
	3	23.0	22.6	**22.8**	39.2	40.4	**39.8**
	4	N.A.[2]	21.8	**21.8**	N.A.[2]	42.6	**42.6**
	5	22.0	22.5	**22.3**	37.3	40.8	**39.1**
			Mean =	**22.3**		Mean =	**40.4**
			Std dev =	**0.5**		Std dev =	**1.6**
700 ppm D4	1	24.3	23.4	**23.9**	40.6	43.3	**42.0**
	2	23.1	23.5	**23.3**	41.2	41.8	**41.5**
	3	23.6	23.8	**23.7**	40.8	41.3	**41.1**
	4	23.0	23.4	**23.2**	41.8	41.4	**41.6**
	5	24.3	23.7	**24.0**	40.3	41.4	**40.9**
			Mean =	**23.6**		Mean =	**41.4**
			Std dev =	**0.3**		Std dev =	**0.4**
PB (0.05%)	1	22.3	21.5	**21.9**	40.4	42.4	**41.4**
	2	20.8	21.2	**21.0**	44.3	43.9	**44.1**
	3	22.1	22.2	**22.2**	40.4	39.9	**40.2**
	4	20.9	21.4	**21.2**	43.9	42.1	**43.0**
	5	21.6	22.2	**21.9**	41.3	39.6	**40.5**
			Mean =	**21.6**		Mean =	**41.8**
			Std dev =	**0.5**		Std dev =	**1.7**

[1]Due to the large number of animals within each group the exposures were conducted in two phases with half the animals in each group exposed in week one (subgroup A) and the second half of the animals exposed in week two (subgroup B).
[2]N.A. = Not Applicable due to malfunctioning chamber sensor.

Table 5

Chamber D4 Vapor Concentration Summary: Phase III

Exposure Concentration	Study Day	Mean Daily D4 Chamber Concentration (ppm)		
		Subgroup A[1]	Subgroup B[1]	Mean
0 ppm D4	1	BLQ[2]	BLQ	
	2	BLQ	BLQ	
	3	BLQ	BLQ	
	4	BLQ	BLQ	
	5	BLQ	BLQ	
7 ppm D4	1	7	7	**7**
	2	6	7	**7**
	3	7	7	**7**
	4	7	7	**7**
	5	7	7	**7**
			Mean =	**7**
			Std dev =	**0**
30 ppm D4	1	31	28	**30**
	2	28	28	**28**
	3	29	29	**29**
	4	30	30	**30**
	5	28	31	**30**
			Mean =	**29**
			Std dev =	**1**
70 ppm D4	1	75	71	**73**
	2	70	73	**72**
	3	69	71	**70**
	4	70	68	**69**
	5	69	62	**66**
			Mean =	**70**
			Std dev =	**3**

[1]Due to the large number of animals within each group the exposures were conducted in two phases with half the animals in each group exposed in week one (subgroup A) and the second half of the animals exposed in week two(subgroup B).
[2]BLQ = below the limit of quantification (approximately 0.04 ppm D4)

Table 5

Chamber D4 Concentration Summary: Phase III
(Continued)

Exposure Concentration	Study Day	Mean Daily D4 Chamber Concentration (ppm)		
		Subgroup A[1]	Subgroup B[1]	Mean
150 ppm D4	1	149	159	**154**
	2	130	152	**141**
	3	149	152	**151**
	4	150	153	**152**
	5	146	154	**150**
			Mean =	**150**
			Std dev =	**5**
300 ppm D4	1	300	316	**308**
	2	301	301	**301**
	3	306	303	**304**
	4	303	268	**286**
	5	303	300	**302**
			Mean =	**300**
			Std dev =	**8**
700 ppm D4	1	708	730	**719**
	2	701	710	**706**
	3	702	692	**697**
	4	704	692	**698**
	5	679	693	**686**
			Mean =	**701**
			Std dev =	**12**

[1]Due to the large number of animals within each group the exposures were conducted in two phases with half the animals in each group exposed in week one (subgroup A) and the second half of the animals exposed in week two(subgroup B).

Appendix C

Individual Animal Clinical Observations

Effects of Octamethylcyclotetrasiloxane (D4) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28-Day Inhalation Study

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
II	Control	A-1	C6388	Normal	P	P	P	P	P	P
II	Control	A-1	C6389	Normal	P	P	P	P	P	P
II	Control	A-1	C6390	Normal	P	P	P	P	P	P
II	Control	A-1	C6391	Normal	P	P	P	P	P	P
II	Control	A-1	C6392	Normal	P	P	P	P	P	P
II	Control	A-1	C6393	Normal	P	P	P	P	P	P
II	Control	A-1	C6394	Normal	P	P	P	P	P	P
II	Control	A-1	C6395	Normal	P	P	P	P	P	P
II	Control	A-1	C6396	Normal	P	P	P	P	P	P
II	Control	A-1	C6397	Normal	P	P	P	NP	NP	NP
				Urine stain, perineal area	NP	NP	NP	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day												
					1	2	3	4	5	6	7	8	9	10	11	12	13
II	Control	B-1	C6398	Normal	P	P	P	P	P	P	P	NP	P	P	P	P	P
				Urine Stain Perineal region	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP
II	Control	B-1	C6399	Normal	P	P	P	P	P	P	P	P	NP	P	P	P	P
				Torn Toe Nail	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP
II	Control	B-1	C6400	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Control	B-1	C6401	Normal	P	P	P	P	P	P	P	P	P	P	P	P	NP
				Urine Stain Perineal Region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P
II	Control	B-1	C6402	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Control	B-1	C6403	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Control	B-1	C6404	Normal	NP	NP	P	P	P	P	P	P	P	P	P	NP	NP
				Front Paw Swollen/Crust	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Urine Stain Prineal Region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P
II	Control	B-1	C6405	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Control	B-1	C6406	Normal	P	P	P	P	P	P	P	P	P	P	P	P	NP
				Urine Stain Perineal Region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P
II	Control	B-1	C6407	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day																									
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
II	Control	C-1	C6408	Normal	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	NP	NP	P	NP	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	P	P	NP	P	NP	NP	NP	NP	NP	NP	NP
II	Control	C-1	C6409	Normal	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	NP	P	NP	NP	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	P	NP	P	P	NP	NP	NP	NP	NP	NP	NP
II	Control	C-1	C6410	Normal	P	P	P	NP	NP	NP	NP	NP	P	P	NP	NP	NP	P	P	P	P	P	P	NP	P	P	P	P	P	NP	NP
				Urine Staining perineal region	NP	NP	NP	P	P	P	P	P	NP	NP	P	P	P	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	P	P
				Clear fluid rt. eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP
II	Control	C-1	C6411	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Control	C-1	C6412	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Control	C-1	C6413	Normal	P	P	NP	P	P	P	P	P	NP	NP	P	P	P	P	NP	P	P	NP	P	NP	P	P	P	NP	NP	NP	P
				Urine Staining perineal region	NP	NP	P	NP	NP	NP	NP	NP	P	P	NP	NP	NP	NP	P	NP	NP	P	NP	P	NP	NP	NP	P	P	P	NP
II	Control	C-1	C6414	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Control	C-1	C6415	Normal	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Control	C-1	C6416	Normal	P	P	P	P	NP	NP	P	P	P	P	P	P	NP	P	NP	P	P	NP	P	P	P	NP	NP	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	P	P	NP	NP	NP	NP	NP	NP	P	NP	P	NP	NP	P	NP	NP	NP	P	P	NP	NP	NP	NP
II	Control	C-1	C6417	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
II	D4 700 PPM	A-2	C6418	Normal	P	P	P	P	P	P
II	D4 700 PPM	A-2	C6419	Normal	P	P	P	P	P	P
II	D4 700 PPM	A-2	C6420	Normal	P	P	NP	NP	NP	NP
				Urine Staining perineal region	NP	NP	P	P	P	P
II	D4 700 PPM	A-2	C6421	Normal	P	P	NP	P	P	P
				Urine Staining perineal region	NP	NP	P	NP	NP	NP
II	D4 700 PPM	A-2	C6422	Normal	P	P	NP	NP	NP	NP
				Urine Staining perineal region	NP	NP	P	P	P	P
II	D4 700 PPM	A-2	C6423	Normal	P	P	P	NP	NP	NP
				Urine Staining perineal region	NP	NP	NP	P	P	P
II	D4 700 PPM	A-2	C6424	Normal	P	P	P	P	P	P
II	D4 700 PPM	A-2	C6425	Normal	P	P	P	P	P	P
II	D4 700 PPM	A-2	C6426	Normal	P	P	P	NP	NP	NP
				Urine Staining perineal region	NP	NP	NP	P	P	P
II	D4 700 PPM	A-2	C6427	Normal	P	P	NP	NP	NP	NP
				Urine Staining perineal region	NP	NP	P	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day												
					1	2	3	4	5	6	7	8	9	10	11	12	13
II	D4 700 PPM	B-2	C6428	Normal	P	P	P	-	P	P	P	P	P	NP	P	P	P
				Urine Stain Perineal region	NP	NP	NP	-	NP	NP	NP	NP	NP	P	NP	NP	NP
II	D4 700 PPM	B-2	C6429	Normal	P	P	P	P	P	P	P	P	P	P	P	NP	NP
				Urine Stain Perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P
II	D4 700 PPM	B-2	C6430	Normal	P	P	P	NP	NP	NP	NP	NP	P	NP	NP	NP	NP
				Urine Stain Perineal region	NP	NP	NP	P	P	P	P	NP	NP	NP	P	P	P
				Porphyrin stain rt. eye	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	P	NP	NP
				Small eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP
				Clear fluid around eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP
II	D4 700 PPM	B-2	C6431	Normal	P	P	P	P	P	P	P	P	P	P	P	NP	P
				Urine Stain Perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP
II	D4 700 PPM	B-2	C6432	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	D4 700 PPM	B-2	C6433	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	D4 700 PPM	B-2	C6434	Normal	P	P	P	NP	P	P	P	P	P	P	NP	NP	NP
				Urine stain perineal region	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	P	P	P
II	D4 700 PPM	B-2	C6435	Normal	P	P	P	P	P	P	P	P	P	P	NP	NP	NP
				Urine stain perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P	P
II	D4 700 PPM	B-2	C6436	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	D4 700 PPM	B-2	C6437	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P

- Clinical observations were inadvertently not recorded

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day																								
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
II	D4 700 PPM	C-2	C6438	Normal	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	NP	P	P	P	P	P	P	NP	P	NP	NP	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP
				Clear fluid rt. eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	P	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP
II	D4 700 PPM	C-2	C6439	Normal	P	P	P	NP	NP	NP	NP	NP	P	P	NP	NP	P	P	P	NP	NP	P	NP	NP	P	NP	P	P	P	NP	P
				Urine Staining perineal region	NP	NP	NP	P	P	P	P	P	NP	NP	P	P	NP	NP	NP	P	P	NP	P	P	NP	NP	NP	NP	NP	NP	NP	NP
				Eye, small, left	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP
II	D4 700 PPM	C-2	C6440	Normal	P	P	P	P	P	P	P	NP	NP	P	P	NP	P	P	P	NP	P	P	NP	P	P	NP	P	P	P	NP	NP
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	P	P	NP	NP	P	NP	NP	NP	P	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	P
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP
				Eye, closed, left	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP
II	D4 700 PPM	C-2	C6441	Normal	P	P	P	P	P	P	P	P	NP	P	P	NP	P	P	P	P	P	P	NP	P	P	P	P	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Porphryn stain; eye; both	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP
II	D4 700 PPM	C-2	C6442	Normal	P	P	P	P	P	P	P	P	NP	NP	NP	NP	P	P	P	P	NP	P	P	P	P	P	NP	NP	NP	NP	NP
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	P	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Clear fluid rt. eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P	P	NP
				Dried fluid rt. eye	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day																									
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
II	D4 700 PPM	C-2	C6443	Normal	P	P	P	NP	P	P	P	P	P	NP	NP	NP	P	P	P	P	NP	P	P	P	P	NP	P	P	NP	P	P
				Urine Staining perineal region	NP	NP	NP	P	NP	NP	NP	NP	NP	P	P	P	NP	NP	NP	NP	P	NP	NP	NP	NP	P	NP	NP	NP	NP	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP
II	D4 700 PPM	C-2	C6444	Normal	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	NP	P	NP	P	P	P	P	P	P	NP	NP
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Implant site scab	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P
II	D4 700 PPM	C-2	C6445	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
II	D4 700 PPM	C-2	C6446	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	NP	P	P	P	NP	P	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	P	NP	NP	NP	P	NP	NP	NP	NP
II	D4 700 PPM	C-2	C6447	Normal	P	P	P	P	P	P	P	P	P	NP	NP	P	P	P	P	P	P	NP	NP	NP	P	P	NP	P	NP	P	P
				Urine Staining perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P	NP	NP	NP	NP	NP	NP	P	P	P	NP	NP	NP	NP	P	NP	NP
				Clear fluid both eyes	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	P	NP	NP

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
II	Phenobarbital	A-3	C6448	Normal	P	P	P	P	P	P
II	Phenobarbital	A-3	C6449	Normal	P	P	P	P	P	P
II	Phenobarbital	A-3	C6450	Normal	P	P	NP	NP	NP	NP
				Urine staining perineal region	NP	NP	P	P	P	P
II	Phenobarbital	A-3	C6451	Normal	P	P	P	P	P	P
II	Phenobarbital	A-3	C6452	Normal	P	P	NP	NP	NP	NP
				Urine staining perineal region	NP	NP	P	P	P	P
				Porphyrin staining both eyes	NP	NP	NP	NP	NP	P
II	Phenobarbital	A-3	C6453	Normal	P	P	P	P	P	P
II	Phenobarbital	A-4	C6454	Normal	P	P	P	P	P	P
II	Phenobarbital	A-3	C6455	Normal	P	P	P	NP	P	P
				Urine staining perineal region	NP	NP	NP	P	NP	NP
II	Phenobarbital	A-3	C6456	Normal	P	P	P	NP	P	P
				Urine staining perineal region	NP	NP	NP	P	NP	NP
II	Phenobarbital	A-3	C6457	Normal	P	P	P	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day												
					1	2	3	4	5	6	7	8	9	10	11	12	13
II	Phenobarbital	B-3	C6458	Normal	P	P	NP	P	P	P	P	P	P	P	P	P	P
				Porphyrin Staining both eyes	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	B-3	C6459	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	B-3	C6460	Normal	P	P	P	NP	P	P	P	P	P	P	P	P	P
				Porphyrin Staining both eyes	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	B-3	C6461	Normal	P	P	P	P	P	P	NP	P	P	P	P	P	P
				Urine staining ,perineal area	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP
II	Phenobarbital	B-3	C6462	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	B-3	C6463	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	B-3	C6464	Normal	P	P	P	NP	P	P	P	P	P	P	P	P	P
				Porphyrin stain both eyes	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	B-3	C6465	Normal	P	NP	NP	NP	NP	NP	NP	P	P	P	P	P	P
				Porphyrin stain rt. eye	NP	NP	P	P	P	P	NP	NP	NP	NP	NP	NP	NP
				Porphyrin stain both eyes	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Urine stain,perineal,abdomen region	NP	NP	NP	NP	P	P	P	NP	NP	NP	NP	NP	NP
II	Phenobarbital	B-3	C6466	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	B-3	C6467	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day																									
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
II	Phenobarbital	C-3	C6468	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	C-3	C6469	Normal	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P
				Urine stain perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	C-3	C6470	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Phenobarbital	C-3	C6471	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	C-3	C6472	Normal	P	P	NP	NP	NP	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
				Porphyrin stain both eyes	NP	NP	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
				Urine stain perineal region	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	C-3	C6473	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
II	Phenobarbital	C-3	C6474	Normal	P	P	P	P	P	P	P	P	P	P	NP	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
				Urine stain perineal region	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	C-3	C6475	Normal	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
				Porphyrin stain rt. eye	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	C-3	C6476	Normal	P	P	P	P	P	P	P	NP	P	NP	P	NP	P	P	NP	NP	NP	NP	NP	NP	P	P	P	NP	P	P	P
				Urine stain perineal region	NP	NP	NP	NP	NP	NP	NP	P	NP	P	NP	P	NP	NP	P	P	P	P	P	P	NP	NP	NP	P	NP	NP	NP
				Sl. Porphyrin stain	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
II	Phenobarbital	C-3	C6477	Normal	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	Control	A-1	C6890	Normal	P	P	P	P	P	P
III	Control	A-1	C6891	Normal	P	P	P	P	P	P
III	Control	A-1	C6892	Normal	P	P	P	P	P	P
III	Control	A-1	C6893	Normal	P	P	P	P	P	P
III	Control	A-1	C6894	Normal	P	P	P	P	P	P
III	Control	B-1	C6930	Normal	P	P	P	P	P	P
III	Control	B-1	C6931	Normal	P	P	P	P	P	P
III	Control	B-1	C6932	Normal	P	P	P	P	P	P
III	Control	B-1	C6933	Normal	P	P	P	P	P	P
III	Control	B-1	C6934	Normal	P	P	P	P	P	P

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	D4 7PPM	A-2	C6895	Normal	P	P	P	P	P	P
III	D4 7PPM	A-2	C6896	Normal	P	P	P	P	P	P
III	D4 7PPM	A-2	C6897	Normal	P	P	P	P	P	P
III	D4 7PPM	A-2	C6898	Normal	P	P	P	P	P	P
III	D4 7PPM	A-2	C6899	Normal	P	P	P	P	P	P
III	D4 7PPM	B-2	C6935	Normal	P	P	P	P	P	NP
				Urine stain perineal region	NP	NP	NP	NP	NP	P
III	D4 7PPM	B-2	C6936	Normal	P	P	P	P	P	P
III	D4 7PPM	B-2	C6937	Normal	P	P	P	P	P	P
III	D4 7PPM	B-2	C6938	Normal	P	P	P	P	P	P
III	D4 7PPM	B-2	C6939	Normal	P	P	P	P	P	P

P=Present
= Not Present

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
	D4-70 PPM				1	2	3	4	5	6
III	D4-70 PPM	A-3	C6900	Normal	P	P	P	P	P	P
III	D4-70 PPM	A-3	C6901	Normal	P	P	P	P	P	P
III	D4-70 PPM	A-3	C6902	Normal	P	P	P	P	P	P
III	D4-70 PPM	A-3	C6903	Normal	P	P	P	P	P	P
III	D4-70 PPM	A-3	C6904	Normal	P	P	P	P	P	P
III	D4-70 PPM	B-3	C6940	Normal	P	P	P	P	P	P
III	D4-70 PPM	B-3	C6941	Normal	P	P	P	P	NP	NP
				Urine stain perineal region	NP	NP	NP	NP	P	P
III	D4-70 PPM	B-3	C6942	Normal	P	P	P	P	P	P
III	D4-70 PPM	B-3	C6943	Normal	P	P	P	P	P	P
III	D4-70 PPM	B-3	C6944	Normal	P	P	P	P	P	P

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	D4-150 PPM	A-4	C6905	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-4	C6906	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-4	C6907	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-4	C6908	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-4	C6909	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-4	C6945	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-4	C6946	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-4	C6947	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-4	C6948	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-4	C6949	Normal	P	P	P	P	P	P

P=Present
NP= Not Present

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	D4-150 PPM	A-5	C6910	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-5	C6911	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-5	C6912	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-5	C6913	Normal	P	P	P	P	P	P
III	D4-150 PPM	A-5	C6914	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-5	C6950	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-5	C6951	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-5	C6952	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-5	C6953	Normal	P	P	P	P	P	P
III	D4-150 PPM	B-5	C6954	Normal	P	P	P	P	P	P

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	D4-300 PPM	A-6	C6915	Normal	P	P	P	P	P	P
III	D4-300 PPM	A-6	C6916	Normal	P	P	P	P	NP	P
				Urine stain perineal region	NP	NP	NP	NP	P	NP
III	D4-300 PPM	A-6	C6917	Normal	P	P	P	P	P	P
III	D4-300 PPM	A-6	C6918	Normal	P	P	P	P	P	P
III	D4-300 PPM	A-6	C6919	Normal	P	P	P	P	P	P
III	D4-300 PPM	B-6	C6955	Normal	P	P	P	P	P	P
III	D4-300 PPM	B-6	C6956	Normal	P	P	P	P	P	P
III	D4-300 PPM	B-6	C6957	Normal	P	P	P	P	P	P
III	D4-300 PPM	B-6	C6958	Normal	P	P	P	P	P	P
III	D4-300 PPM	B-6	C6959	Normal	P	P	P	P	P	P

P=Present
= Not Present

Appendix C

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	D4-700 PPM	A-7	C6920	Normal	P	P	P	P	NP	NP
				Urine stain perineal region	NP	NP	NP	NP	P	P
III	D4-700 PPM	A-7	C6921	Normal	P	P	P	P	P	P
III	D4-700 PPM	A-7	C6922	Normal	P	P	P	P	P	P
III	D4-700 PPM	A-7	C6923	Normal	P	P	P	P	P	P
III	D4-700 PPM	A-7	C6924	Normal	P	P	P	P	NP	NP
				Urine stain perineal region	NP	NP	NP	NP	P	P
III	D4-700 PPM	B-7	C6960	Normal	P	P	P	P	P	P
III	D4-700 PPM	B-7	C6961	Normal	P	P	P	P	P	P
III	D4-700 PPM	B-7	C6962	Normal	p	p	p	p	p	p
III	D4-700 PPM	B-7	C6963	Normal	P	P	P	P	P	P
III	D4-700 PPM	B-7	C6964	Normal	P	P	P	NP	NP	NP
				Urine stain perineal region	NP	NP	NP	P	P	P

Phase	Treatment Group	Sub-Group	Animal Number	Clinical Sign	Study Day					
					1	2	3	4	5	6
III	Phenobarbital	A-8	C6925	Normal	P	P	P	P	NP	NP
				Porphyrin stain both eyes	NP	NP	NP	NP	P	P
III	Phenobarbital	A-8	C6926	Normal	P	P	P	P	P	P
III	Phenobarbital	A-8	C6927	Normal	P	P	P	P	P	P
III	Phenobarbital	A-8	C6928	Normal	P	P	P	P	P	P
III	Phenobarbital	A-8	C6929	Normal	P	P	P	NP	NP	P
				Porphyrin stain left eye	NP	NP	NP	P	P	NP
III	Phenobarbital	B-8	C6965	Normal	P	P	P	P	P	P
III	Phenobarbital	B-8	C6966	Normal	P	P	P	P	P	P
III	Phenobarbital	B-8	C6967	Normal	P	P	P	NP	NP	NP
				Porphyrin stain right eye	NP	NP	NP	P	P	P
III	Phenobarbital	B-8	C6968	Normal	P	P	NP	NP	NP	NP
				Porphyrin stain both eyes	NP	NP	P	P	NP	NP
				Porphyrin stain right eye	NP	NP	NP	NP	P	P
III	Phenobarbital	B-8	C6969	Normal	P	P	NP	NP	P	P
				Porphyrin stain both eyes	NP	NP	P	NP	NP	NP
				Porphyrin stain right eye	NP	NP	NP	P	NP	NP

P=Present
NP= Not Present